ASPECTS OF GENETICS OF HOST PATHOGEN INTERACTION IN *POPULUS - MELAMPSORA MEDUSAE* SYSTEM

By

C. S. PRAKASH

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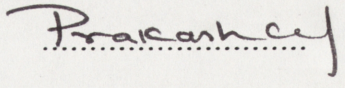
STATEMENT OF ORIGINALITY

Except where acknowledged this thesis is my original work



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ABBREVIATIONS USED IN THE THESIS

ANOVA	-	Analysis of Variance
°C	-	Celsius (Temperature)
c.	-	circa, around, about
cv.	-	cultivar
cvs	-	cultivars
cm	-	centimetre
D F	-	Degrees of Freedom
EMS	-	Ethyl Methane Sulphonate (chemical mutagen)
F1	-	first filial generation
GA	-	Gibberellic Acid
Gy	-	Gray (Irradiation) = 0.1 kR
Gen	-	Generations
h	-	hour
HT	-	High Temperature
IPF	-	Incubation Period to Flecking (the initial symptoms of disease)
IT	-	Infection Type
kR	-	kilo Rad (Irradiation)
L	-	Litre
log	-	logarithm to the base e
LP1	-	Latent Period to production of first uredinium
LP50	-	Latent Period to production of 50% uredinia
LSD	-	Least Significant Difference
LT	-	Low Temperature
max.	-	maximum
mg	-	milligram
mm	-	millimetre
min.	-	minimum
M.S.S.	-	Mean Sum of Squares
u	-	micro
uEm-2s-1	-	micro Einsteins, per square metre, per second (light intensity)
NS	-	Not Significant
P	-	Probability level
R	-	Resistant cultivar
R2	-	coefficient of determination
r	-	correlation coefficient
RH	-	Relative Humidity
S	-	Susceptible
Sec	-	Seconds
SED	-	Standard Errors of Difference
sign.	-	significant
ULD	-	Uredinia produced per Leaf Disk (1.70 cm^2)
UPD	-	Uredinia Produced per leaf disk per Day
USM	-	Urediniospores produced per sq. mm of leaf area
USU	-	Urediniospores produced per Uredinium
V.R.	-	Variance Ratio
w/v	-	weight by volume
>	-	more than
<	-	less than

ABSTRACT

Changes, due to variability and selection, in virulence and aggressiveness of Melampsora medusae Thum., a cause of leaf rust in poplar, and the inheritance of resistance to this pathogen in the hybrids of Populus deltoides Marsh. cultivars were investigated. The study, which was conducted under controlled environmental conditions, employed detached leaf disks (1.70 cm2) of various cultivars raised in a rust-free glasshouse, inoculated with a specific inoculum (urediniospores) of M. medusae and subsequently incubated in Petri dishes kept in growth chambers. The host/pathogen reactions were assessed on a qualitative (basis infection type), or quantitative (various traits of both timing and severity of disease, including time to flecking and production of uredinia, uredinial density, numbers of urediniospores produced and disease progress), scale.

Mutation to virulence on a resistant P. deltoides cv. T-173 was induced by treatment of urediniospores of an avirulent race (5A) of M. medusae with gamma irradiation. The frequency of mutation was maximal (1.69%) at 400 Gy. Natural mutation for virulence on this cultivar in race 4A was also observed. In a preliminary study, three races of M. medusae differed in their sensitivity to increasing levels of irradiation. These results are consistent with a gene-for-gene basis of the relationship between M. medusae and poplars, as the resistance gene in cv. T-173 was countered by mutation to virulence of these races.

When inoculated on a series of poplar cultivars, the radiation induced (5), and natural (2), virulent mutant isolates had a considerably wider virulence spectrum (host range) when compared to their respective wild types. However on one cultivar (P. deltoides cv. 7-2), four of the five induced mutant isolates had lost their virulence. On all the cultivars, although there were differences in aggressiveness among the mutants, such differences were greater between the mutants and wild types.

On a susceptible cultivar (P. x euramericana (Dode) Guinier cv. I-488), on which the virulent mutants can arise in nature, the virulent mutant isolates (both radiation induced and natural) were less aggressive for traits of disease severity but had a relatively shorter latent period, when compared to the wild type. Possibly, on susceptible cultivars, the increased virulence spectrum in the pathogen is associated with reduced aggressiveness, while the fitness of the new virulent mutants on the resistant cultivar depends on the genetic background of the host.

When two races of M. medusae were cultured separately for 11 serial generations (asexual) on cv. I-488, an increase in aggressiveness of these races, with an increase in the number of generations of culture, was evident on this and another cultivar (P. x euramericana cv. I-214). This indicated the occurrence of host selection pressure on the pathogen biotypes with enhanced aggressiveness, and thus the gradual erosion of quantitative components of resistance. On cv. I-488, the magnitude of increase in uredinial and urediniospore numbers of the latter generation isolates was slightly higher in the isolates that were irradiated (400 Gy) prior to the commencement of the serial culture. This suggested the possible occurrence of mutations in, and thus a genetic basis of, observed quantitative traits in the pathogen.

In a similar study, when a virulent race (radiation induced or natural) of M. medusae was serially cultured for 11 generations on cv. I-488, although isolates increased their aggressiveness on this cultivar over generations, they exhibited reduced aggressiveness (most traits) when inoculated on cv. T-173. Similarly, those isolates cultured on cv. T-173, had decreasing trends of aggressiveness on cv. I-488. Such a negative association of aggressiveness trends in the pathogen races on certain cultivars when serially cultured on other cultivars has implications in disease control by creating host heterogeneity and thus effecting a disruptive selection on the population of the pathogen.

When an virulent race was cultured in mixture with an avirulent race (1:1) on a susceptible cultivar (I-488), the aggressiveness of the race mix decreased on the resistant cultivar (T-

173), with an increase in the number of generations of culture. This suggested that, on the susceptible cultivar, the competitive ability of the virulent race (with wider virulence spectrum) was reduced when compared to avirulent race (with narrower virulence spectrum). Additionally, the aggressiveness of the race mix on the susceptible cultivar was less compared to that of pure races, suggesting some form of antagonism occurring between the races when mixed. This supports the desirability to promote or maintain polymorphism in the pathogen population.

Race 4A of M. medusae is avirulent on P. deltoides cv. W-79/307 when incubated at high temperature (26°C/100 $uEm^{-2}s^{-1}$) or high light intensity (17°C/ 700 $uEm^{-2}s^{-1}$) but virulent at low temperature and light intensity (17°C/100 $uEm^{-2}s^{-1}$). When this race was serially cultured at increasing temperatures (17, 20, 23 and 26°C), isolates that were apparently adapted to each of these regimes, and thus virulent at higher temperatures, were obtained. This indicates considerable plasticity in the pathogen. Such isolates, particularly from low temperatures, exhibited specificity to their 'own' evironment of incubation, although high temperature selected isolates were aggressive at all temperatures. A similar adaptation in this race to increasing light intensities of incubation was observed. It is suggested that evironment may play an important role in the stability of the pathosystem by favouring variability in the pathogen, as a consequence of spatial and temporal heterogeneity of the physical environmental variables.

When sets of leaf disks of cv. W-79/307 inoculated with a temperature-sensitive race of the pathogen, were reciprocally transferred daily between compatible and incompatible temperatures, the recognition of incompatible reaction appeared to be an active, irreversible, and more rapid (c. 15 h) phenomena when compared to compatibility, which was reversible and relatively slower (c. 4 days). Further, the temperature activated incompatibility, once triggered, was epistatic to subsequent potential compatibility of this cultivar with a temperature-non-sensitive race of the pathogen.

Resistance (qualitative) to five races of M. medusae was observed to be simply inherited in the F1 plants of a cross of P. deltoides cultivars. It was hypothesised as dominant (3 races), recessive (1 race) and additive/codominant (1 race), possibly controlled by a single gene or two genes acting in a complementary manner (except with the latter race), depending on the race employed.

In the same group of F1 plants, the quantitative resistance (five traits) to two races (virulent on both parents) appeared to be polygenically inherited. Considerable transgressive segregation and quantitative race-specificity were observed in the progeny. Although additive control of the traits was evident, based on the skewness, the higher resistance in most traits appeared to be recessive to lower resistance. Similar results were observed in a further three crosses of P. deltoides cultivars, however, an interaction of major genes with polygenes was evident in these crosses. A rapid improvement in the level of quantitative resistance and the combining of broad spectrum qualitative resistance (against many races) and high levels of quantitative resistance to some virulent races, should be possible.

Overall the results indicate a potential in this pathogen for considerable diversity in virulence and aggressiveness, and the ability to respond to selection pressures by host and environment. The cultivars exhibit both qualitative and quantitative specificity in resistance to the races or isolates (e.g. virulent mutants) of the pathogen. If confirmed from field studies, these and other observed features of the system, could be exploited in disease management by deployment of appropriate cultivars as mixtures or mosaics. These aspects, including variability in the pathogen for virulence and aggressiveness, the relationship between these parameters, host and environmental selection pressures, the micro-evolution of the pathogen and stability of resistance, are discussed with reference to the experimental results.

PUBLICATIONS BY THE AUTHOR RELATED TO THIS THESIS*

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Prakash, C. S. and Heather, W. A. (1985) Response to gamma irradiation and induced virulent mutation in Melampsora medusae of poplars. Phytopathologische Zeitschrift (in press).

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PAPERS IN PREPARATION

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ABSTRACT/POSTERS/PAPERS PRESENTED AT CONFERENCE

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* The contribution by Dr. W. A. Heather in these papers is that normally made by a supervisor.

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INTRODUCTION

" The gods, to console the Heliades for the Death of their brother Phathon, changed them into poplars"

(adapted from Ovid-Noveau Duhamel-Paris 1804)

CHAPTER 1

INTRODUCTION

1.1. THE GENUS POPULUS

Poplars are important trees, both as commercial plantations and as ornamentals. The genus Populus (Family Salicaceae) has been recommended by the FAO to help meet the increasing timber requirements of the growing world (Anon, 1979) and 'represents a significant component of the world's potential renewable resources for the twenty-first century' (Thielges, 1984). Due to their rapid growth rate, high biomass synthesis and ease of vegetative propagation, poplars have been planted extensively in northern and southern hemispheres (Pryor, 1969; Anon, 1979), for pulp and wood products including veener, light box making material, fibre boards, safety matches etc. A brief description of the taxonomic division of the genus Populus is presented in Appendix 2.

1.2 POPLARS IN AUSTRALIA

Although poplars have been grown in Australia as ornamental trees since mid ninteenth century (Palmberg, 1977), the commercial plantations of poplars are of relatively recent origin (Brown, 1971). The commercial importance of poplars and their potential in Australia are summarised by Pryor (1969) and Pryor and Willing (1982). In Australia, although the present area under commercial poplar cultivation is less than 10 000 ha, breeding of poplars with high productivity and disease resistance could possibly result in an increase in area under poplar cultivation (Pryor and Willing, 1982).

1.3 DISEASES OF POPLARS

More than 110 organisms have been reported as pathogenic on Populus (Berbee, 1964) and descriptions of some of the important diseases are available (Anon, 1979, Thielges and Land, 1976). However, it has been generally accepted that leaf rust is, by far, the most important disease of poplars in northern America, Europe and Australasia (Muhle Larsen, 1970; Walker et al, 1974; Pryor, 1976; Thielges, 1984).

1.4 LEAF RUST OF POPLARS

Leaf rust in poplars, caused by a number of Melampsora species (Melampsoracae, Uredinales, Basidiomycetes), is a widely distributed disease, causing considerable losses throughout the poplar growing regions of the world (Anon, 1979; Ziller, 1965). The three species, Melampsora medusae Thum., Melampsora larici-populina Kleb., and Melampsora allii-populina Kleb. have been recognised as economically, the most important species which infect sections Aigeiros and Tacamahaca of Populus and their hybrids (Shain, 1976). Melampsora medusae (Plate 1), first described by Thuemen (1878), is widely distributed in the Americas and Australasia and causes considerable reduction in growth (up to 65%) and is also a threat to fibre production (Toole, 1967; Schipper and Dawson, 1974; Thielges and Adams, 1975; Widin and Schipper, 1981). The attack of leaf rust on ornamental plants reduces their aesthetic value (Heather and Sharma, 1977).

The initial attack of rust on poplar occurs in early/mid summer, and when environmental conditions are favourable for the rapid development of the pathogen, heavy infection is obvious by the end of summer. This causes extensive necrosis in the leaves resulting in premature leaf abcission, and sometimes, complete defoliation of the susceptible hosts. Such early defoliation, apart from causing significant reduction in growth and yield, may also predispose the affected trees to secondary problems such as damage by early frosts (Peace, 1962) and to other diseases like Dothichiza and Cytospora cankers (Anon, 1979). Powdery masses of bright orange to yellow

(Reproduced with permission from Singh, 1983).

Plate 1 A young plant of *P. X euramericana* cv. *I-488* bearing uredinia (yellow pustules) of *M. medusae* on its leaves.

urediniospores (Plate 1) are seen abundantly, and sometimes exclusively on the abaxial surface of the infected leaves. Telia may appear late in summer. The Melampsora spp. causing leaf rust of poplars (except M. aecidioides on Populus alba L., Van Vloten, 1946) are heteroecious (i.e. require an alternate host species to complete their life cycle) and macro-cyclic (i.e. produce all spore types typical of rust fungi e.g. aecio-, uredinio-, teleuto-, basidio-, and pycniospores) (Arthur and Cummins, 1962). The life cycle of a typical macro-cyclic Melampsora, causing leaf rust of poplars, is presented in Fig. 1.1. In New Zealand the M. medusae has been recorded in the field on Larix spp. and Pinus radiata D. Don. However in Australia, the sexual stage has not been recorded on any of the accepted alternate hosts, thus the present study is confined to the uredinial monocycle.

1.5 LEAF RUSTS IN AUSTRALIA

Two species, M. medusae of northern American origin and M. larici-populina of European origin which are the principal cause of leaf rusts of poplars in Australasia are relatively recent introductions (Walker et al, 1974, Van Kraayernoord, 1974). Soon after its introduction to Australia in 1972, M. medusae spread to most states in Australia and subsequently to New Zealand. Together with M. larici-populina, introduced in 1973, it caused wide spread damage on many cultivars of poplars (Walker et al, 1974) and certain clones were virtually eliminated from culture in these two countries (Thielges, 1984). The details on etiology, epidemiology, ecology and management options of Melampsora rusts have been presented by Taris (1968), Shain (1976), Siwecki et al (1982) and Heather and Chandrashekar (1982).

1.6 PREVIOUS STUDIES ON HOST-PATHOGEN INTERACTIONS IN MELAMPSORA MEDUSAE

Selection and breeding for disease resistance has been recommended as the major component of the management of leaf rust caused by Melampsora species (Muhle larsen, 1970; Van Karaayenrood, 1974; Shain, 1976; Thielges and Land, 1976; Gremmen, 1980; Willing and Pryor, 1982; Heather and Chandrashekar, 1982; Thielges, 1984). Such an approach is promising

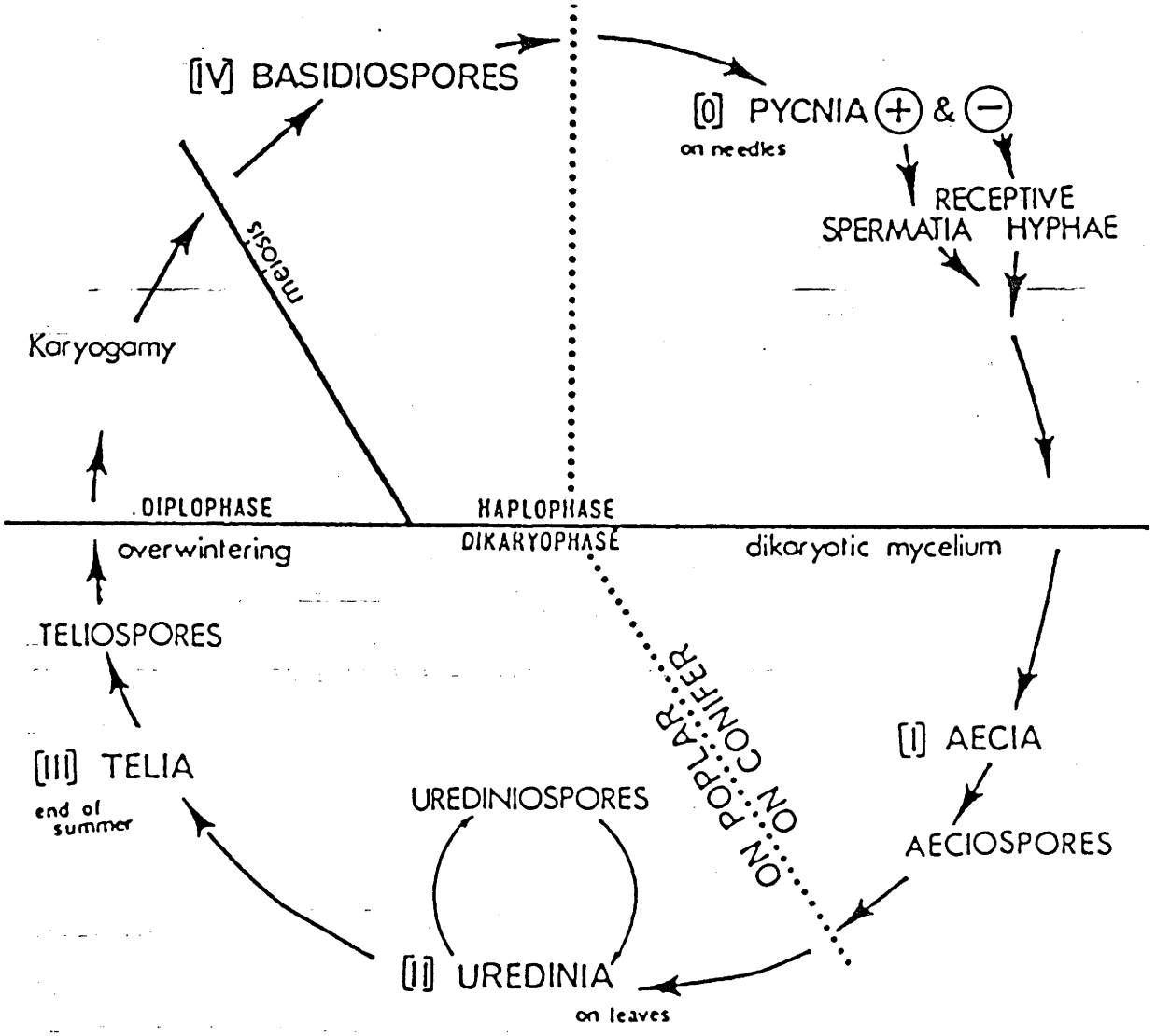


Figure 1.1 Life cycle of typical poplar leaf rust (Reproduced from Omar, 1978).

in poplars due to the availability of resistant sources, ease of clonal propagation, and is recommended also for economic, management and ecological reasons.

In a field study, Eldridge et al (1973) observed that a collection of cultivars of poplars, which was introduced into Australia from different provenances of USA (Brown, 1971), exhibited differences in the disease reaction ranging from complete susceptibility to immunity, to M. medusae. Pryor (1976) has detailed early efforts to identify and the use of sources of resistance to M. medusae in Australia. Sharma and Heather (1976) reported the occurrence of six physiologic races in M. medusae and five races in M. larici-populina in Australia. Subsequently, Sharma and Heather (1977) reported M. medusae on certain clones of Populus alba L. which had been previously considered immune to this pathogen, and attributed it to the possible occurrence of a new physiologic race which may have arisen due to a mutation. Later, the latent period of the pathogen on this clone was found to decrease from 35 to 8 days, suggesting the quantitative adaptation of the new mutant race to its new host (Singh, 1983). Subsequently, Singh and Heather (1982b) reported the occurrence of numerous physiologic races of this pathogen and thus emphasised the need for precise studies on pathogen variability to effectively monitor the stability of resistance. After a series of studies (using detached leaf disks) involving races of M. medusae and cultivars of poplars, Singh and Heather (1982b and 1983) observed considerable differences in the reaction of cultivars of poplars to races of M. medusae leaf rust, and recorded both qualitative and quantitative host-pathogen specificity. Further, such an interaction was sensitive to environmental variables like temperature, light intensity and photoperiod.

1.7 THE NEED FOR STUDIES ON GENETIC ASPECTS OF HOST-PATHOGEN INTERACTION IN M. MEDUSAE/POPULUS SYSTEM

Although there are several reports on the variability in virulence and aggressiveness of Melampsora leaf rusts on poplars, there is no information on the nature of changes in these traits. In some agricultural plants, selection and breeding for resistance to diseases

has been a recurring excercise, mainly due to the continuous and seemingly endless variation in virulence and aggressiveness in pathogens. This has resulted in the 'boom and bust cycle'. Thus it is important to understand the source, nature and extent of variability in the pathogen. This variation and selection in nature for improved parasitic fitness in the pathogen would have profound implications in disease management using resistant cultivars. Also, due to the environmental sensitivity of the system (section 1.6), it would be of significance to understand the impact of environmental variables (e.g. temperature, light intensity) on the dynamics of the pathogen population and their interaction with the host. Finally, knowledge of inheritance of resistance (both qualitative and quantitative) is useful in devising appropriate breeding strategies, and would have relevance in increasing the effectiveness and stability of resistance. A holistic understanding of the three components of the disease triangle (host-pathogen-environment), helps to ensure the stability of host-pathogen relationship. This is more pertinent in a perennial crop because of the relatively uniform host genotype over time.

Genetic studies in this system have some major logistic difficulties, viz. 1) The sexual stage of this pathogen has not been reported in Australia and thus hybridization among biotypes is not possible (the major source of variation in the pathogen in field is probably mutation and other asexual mechanisms). 2) Poplars are dioecious perennials with a long generation interval (7-8 years), hence they are impossible to self and time consuming to backcross. Precise and extensive genetic analysis beyond the F1 generation of the host is not feasible within the doctoral time framework. Thus, a degree of speculation has been indulged to discuss the present results.

Some of the constraints of heterogeneity of the host, pathogen and environment were overcome by employing an *in vitro* system using, uniform detached leaves and leaf disks from plants grown under controlled conditions, pure cultures (mono-urediniospore or mono-uredinium derived isolates) of the pathogen, and controlled physical environment for incubation. Hence factors of experimental error were reduced to a minimum. Preliminary results from laboratory studies,

obtainable in a relatively short time, have significance, particulary due to the high labor costs of efficient programmes in the field in Australia. Day (1974) has emphasised the need for such precise controlled experiments in genetic studies of the host-pathogen interaction.

Due to the breadth of the aspects of the study, for convenience, the thesis has been divided into three major sections. Thus, 'introduction' has been presented at three hierarchial levels, while 'discussion' is presented at two levels. Minor repetition of some concepts between levels has been unavoidable to retain the independence and relevance of this discussion. The list of abbreviations used in the thesis has been provided in the beginning of the thesis, and a glossary of some terms adopted has been presented in Appendix 1.

Section I focusses on the variability in M. medusae for virulence and aggressiveness, the relationship between these two traits and between aggressiveness traits of the races on different cultivars, the host selection for aggressiveness traits, and the implications of these observations in disease epidemiology. Section II examines the response of a pathogen population to variations in temperature and light intensity, and considers the implications of such environmental sensitivity in this pathosystem. Section III deals with the inheritance of qualitative and quantitative resistance in certain poplar cultivars to different races of M. medusae and examines the relevance of the results in the context of disease management through breeding.

Due to the paucity of the literature on genetic studies of host-pathogen interaction in tree crops, much of the cited literature is from agricultural pathosystems.

Note: The terms 'pathogen' and 'host' used in the following section (1.8) refer to M. medusae and poplars, respectively.

1.8 THE OBJECTIVES OF STUDIES REPORTED IN THIS THESIS

I Investigate the occurrence of variability for virulence and

aggressiveness, and examine the possible interrelationship between these.

1. To study the possibility of inducing virulent mutations in races avirulent on a specific resistant cultivar.

2. To compare the virulent mutants and the wild types for their reaction on a range of cultivars (virulence spectrum).

3. To study the relationship, between increased virulence spectrum and the aggressiveness, of the virulent mutants and the wild types.

4. To examine whether directional selection is exercised by the host towards increased aggressiveness on the pathogen.

5. To examine the possible occurrence of association and disassociation of aggressiveness by the races on different cultivars, and evaluate the competitive ability in the mixtures of races differing in their virulence spectrum.

II Investigate the role of changing environmental variables (temperature and light intensity) on the host pathogen relationship.

6. To examine the extent of selection pressure imposed by changing temperatures and light intensities, on virulence and aggressiveness of the population of a race on a cultivar.

7. To study the timing of activation of temperature induced in/compatibility and its possible implication in induced resistance.

III Study the genetics of resistance in the host to the pathogen.

8. To study the inheritance of qualitative resistance in the host to a range of pathogen races.

9. To evaluate the inheritance in compatible host genotypes of quantitative components of resistance to the pathogen .

CHAPTER 2

GENERAL MATERIALS AND METHODS

2.1 INTRODUCTION

The general techniques and materials used throughout the study are described in this chapter. However the specific and minor variations pertinent to each individual study are described in relevant chapters.

An *in vitro* technique was employed in the study, to produce the inoculum of the urediniospores on detached leaves of poplars and leaf disks of the cultivars were used for the experimental studies. The merits and deficiencies of such a system have been discussed by Chandrashekar (1981) and Singh (1983). There are several reports which describe the comparability of such an *in vitro* system with intact plants in the field for studies on host-pathogen interaction (Shain, 1976; Wilcoxson, 1976; Eskes, 1983b; Rappilly *et al* 1981) and in poplar leaf rust situation (Denbnovetskii and Basova, 1980). The present system employed a high degree of replication and was conducted under controlled laboratory conditions. Thus, although extrapolation of results obtained from such a system to the epidemiology in field conditions can only be tentative and must be made with caution, it permits assessment of the precise influence of the treatment variables on the pathosystem in absence of the potentially masking flux of conditions in the field.

2.2 THE HOST

2.2.1 SOURCE OF CULTIVARS OF POPULUS SPECIES

2.2.1.1 THE PROPAGATION OF THE CULTIVARS : Shoot cuttings of all the cultivars used in the study were obtained from the Departments of Forestry and Botany, of The Australian National University. Information on the origin and history of the cultivars is presented in Appendix 2.

2.2.1.2 MAINTENANCE OF THE CLONAL PLANTS : The cultivars of Populus species were vegetatively propagated as clones. Cuttings (c. 10 cm long, 1-2 cm diam.), each having 2 to 3 buds, were initially disinfected (1% sodium hypochlorite solution) for two minutes, washed twice in water, the distal end dipped in indole acetic acid (50mg/L) to stimulate rooting and planted in fully expanded, water soaked Jiffy-7 700® peat pellet (Jiffy Products Ltd, Grorud, Norway). The cuttings were kept at 20±1 °C in a mist chamber with c. 80% relative humidity, regulated by an automatic sprinkler system. After four weeks, when sufficient rooting had occurred, the plants were transferred into 15 cm (diam.) plastic pots containing vermiculite and perlite (1:1) (Australian Gypsum, Australia) and subsequently maintained in a rust proofed glass house (Plate 2.1d) (double door, wet pad filter-air inlet) at 20 ±3 °C and a photoperiod of 16 h light/8 h dark, regulated by an automatic timing device. Leaf rust contamination of these plants in the glass house was very rare and eliminated by roguing. The pots were watered twice daily, by automatic drip irrigation, but additional supplementary watering was required during summer. The nutrients were supplied through Osmocote® (Sierra Chemical Co, USA), a slow release fertiliser (N:P:K, 18:2.6:10), applied once in 2-3 months and a liquid fertiliser, Aquasol® (c. 300 mg/L) (Hortico Pty. Ltd., Australia; contains apart from NPK eight other essential elements) applied once a week.

Occasionally white flies and two-spotted-mites were noticed on the plants and were controlled by applying Temik® 10G (Aldicarb, Union Carbide, Australia) and Lane Dipel® HG, a bacterial insecticide (Bacillus thuriengisis var. alesti) (Roche-Maag, Australia). Residual toxicity of these chemicals were kept to a minimum by applying them only when essential and collecting the leaf samples for inoculations at least two weeks after the previous application.

The plants were repotted at least once in 12 months and shoots were cut back periodically to ensure the supply of leaves of uniform age and maturity (c. 3 months). The positions of the pots were frequently interchanged to minimise micro-climatic influences.

2.2.2 SELECTION AND PREPARATION OF LEAVES AND LEAF DISKS FOR INOCULATION

2.2.2.1 SELECTION OF LEAVES OF UNIFORM AGE AND MATURITY: The position of the leaves on the shoot (age) and the maturity of the shoot on which they are borne have a significant effect on their disease expression to M. larici-populina (Sharma et al, 1980). Therefore, for all the experimental inoculations, a uniform procedure in which leaves were selected only from the central part of shoots of comparable maturity, were followed. However, due to the practical problem involved in synchronising the availability of leaves of a particular maturity, leaf age varied between the experiments upto a maximum of 15 days. However, within an experiment the maturity of leaf samples from a cultivar varied usually by only 2-3 days.

2.2.2.2 TREATMENT OF THE LEAF SURFACE : Certain saprophytic phylloplane fungi may hinder the development of M. larici-populina (Sharma and Heather, 1982), hence the leaves were surface sterilised prior to inoculation with the urediniospores. The freshly harvested leaves were rinsed in 1% sodium hypochlorite solution for 60-90 sec, subsequently washed in three changes of sterile distilled water and dried between folds of sterile blotter sheets. Occasionally, when necessary the leaves were stored briefly (max 1 h) in polythene bags at -4 °C prior to the surface treatment.

2.2.2.3 PREPARATION OF LEAF DISKS : The leaf disks (1.70 cm2) were carefully stamped from the surface treated leaves, employing a spring loaded cork borer(Plate 2.1e), under sterile conditions (Singh and Heather, 1981) and leaf disks with major veins were discarded. With this technique, the variation in leaf area was less than 0.01 cm^2 (Rayner, 1983). Usually, leaf disks were used immediately or occasionally stored briefly on plastic foam soaked with gibberellic acid (10 mg/L) (GA) in sealed plastic Petri dishes at $16_{\pm 1}°C$.

Plate 2.1

a. A stand of Populus deltoides trees infected with Melampsora medusae.

b. Petri dishes, containing leaf segments inoculated with M. medusae, being incubated in control growth cabinets under 'standard environment'.

c. Mature uredinia of M. medusae on detached leaf segments of P. x euramericana cv. I-488, supported on gibberellic acid (GA) solution and located in plastic Petri dishes.


a


b

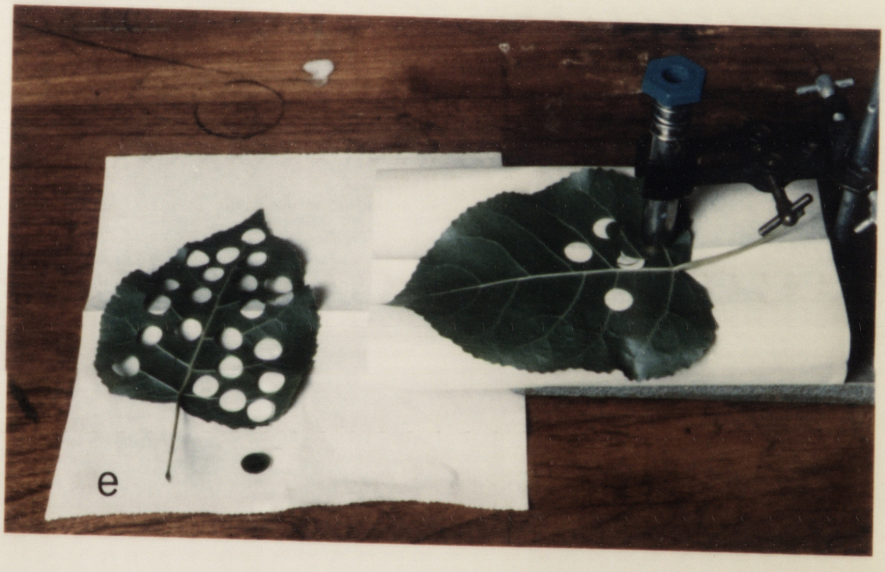

c

Plate 2.1 (Contd.)

d. Clonal plants of poplar cultivars maintained in the rust-free glass house.

e. The leaf disk punching equipment.

f. Infected leaf disks of many cultivars, as they appear towards the end of the monocycle.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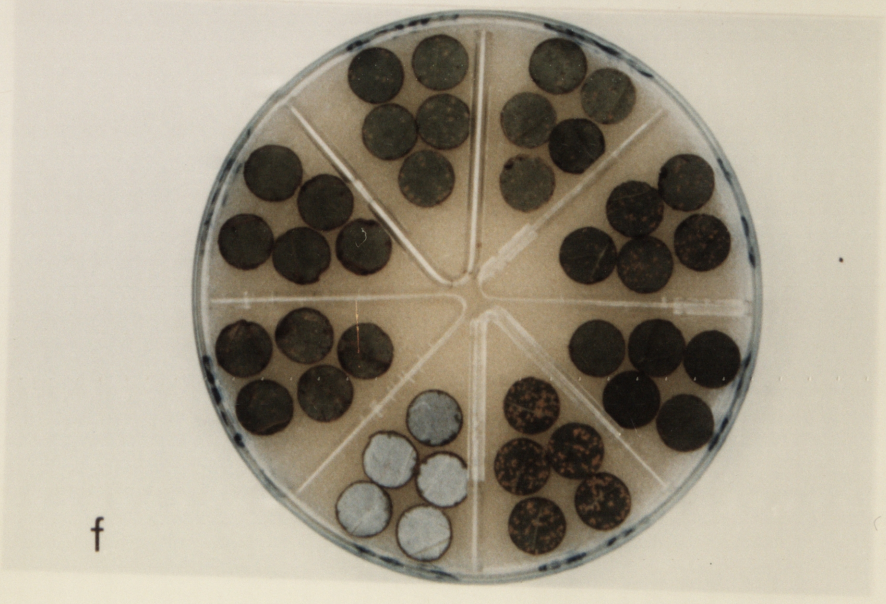

f

2.3 THE PATHOGEN

2.3.1 SOURCE OF UREDINIOSPORES : Many races used in the study (races 1A, 2A, 3A, 4A and 5A) (which were derived from single separate urediniospore; Singh and Heather, 1982b), were obtained from the collection of the Department of Forestry, ANU, and were recognised as qualitatively distinct races (sensu Stakman and Christensen, 1953) by their reaction on differential cultivars. Races 4B, 4B1, 4C, 4M1 and 4M2 were isolated from within race 4A using a leaf replica technique (see Appendix 3). Races 7A and 8A were isolated by the author in the field from an unknown clone of Populus deltoides Marsh. and P. deltoides cv. 60/122, respectively, and are of mono-urediniospore origin. Isolation of single spore cultures using a fine tipped glass needle, were made as detailed by Singh (1983). Race 5M (1-5) is an irradiated mutant series derived from race 5A, isolated by their virulence on P. deltoides cv. T-173, and are mono-uredinial in origin (see Chapter 4). In addition, certain isolates or populations were used from within these races and are described in relevant sections.

2.3.2 COLLECTION AND MAINTENANCE OF UREDINIOSPORES HARVESTED FROM INFECTED LEAVES : Urediniospores were harvested from infected leaves (section 2.5.1) when a majority of the pustules (> 80%) were fully formed, appeared fluffy and exhibited signs of disintegration (Plate 2.1c). The spores were lightly scraped (under sterile conditions, using a sterile scalpel), placed in air-tight plastic vials and dried in vacuo at room temperature over silica gel and P_2O_5 in a glass dessicator (usuallly 24 h each). The drying reduces the moisture content of the fresh spores from c. 80% to c. 20% (Prakash, unpublished), helps maintain the viability of urediniospores, and reduces clumping thus ensuring uniform deposition across inoculations. For experimental inoculations on the leaf disks, the urediniospores were used immediately after drying; otherwise they were placed in vials and stored, in air-tight glass bottles with silica gel in a freezer, at -14 °C. All the races were routinely multiplied on Populus x euramericana (Dode) Guinier cv. I-488 at c. 4 months intervals.

2.4 INOCULATION OF LEAVES AND LEAF DISKS WITH UREDINIOSPORES

2.4.1. INOCULATION OF DETACHED LEAVES FOR MULTIPLICATION OF UREDINIOSPORES : Surface treated (section 2.3.2) leaves or leaf segments, cut to fit within 10 cm (diam.) Petri dishes, were used for general multiplication of the urediniospores (Plate 2.1c). The abaxial surface of the leaves was inoculated evenly by gently dusting with urediniospores using a sterile scalpel, and the inoculated leaves were placed (inoculated side uppermost) in plastic Petri dishes containing 10 ml sterile gibberellic acid solution (GA) (10 mg/L). Occasionally, if hyperparasite (Cladosporium spp.) contamination was noticed, the inoculated leaves were further sterilised after 48 h, to eliminate such contamination (Singh and Heather, 1982).

2.4.2. INOCULATION OF LEAF DISKS : The freshly cut leaf disks from a particular cultivar were initially shaken in a polythene bag to ensure randomisation, and replicate disks and a few cover glasses were located randomly on a template in the revolving base of a spore settling tower (Plate 2.2; Fig. 2.1); the top of the template was lined with a wet sterile paper (soaked with GA) with specific random markings for position of replicate leaf disks of different cultivars.

The spore settling tower was constructed of Perspex (inside dimensions 23.5 x 23.5 x 116.5 cm length, breadth and height respectively) and is illustrated in Fig. 2.1 and Plate 2.2. A weighed quantity of fresh, dry urediniospores (5, 4 or 3 mg) was placed in the spore gun and blown into the tower employing an air-current of 70 Kpa for 30 seconds. The sliding lid at the base of the tower was closed initially for 10 sec to collect any spore clumps deposited and subsequently opened. The urediniospores were allowed to settle uniformly on leaf disks and cover glasses for five minutes. The number of spores/unit area was recorded on the coverglasses.

The germinative potential of the urediniospores was assessed by incubating the inoculated coverglasses in the glass Petri dishes containing plastic foam soaked with sterile distilled water (99-100% RH), and the germination count was taken after 24 h. The spore was considered to have germinated if the germtube was as long as it was broad (Omar, 1978). Between successive inoculations, the tower was

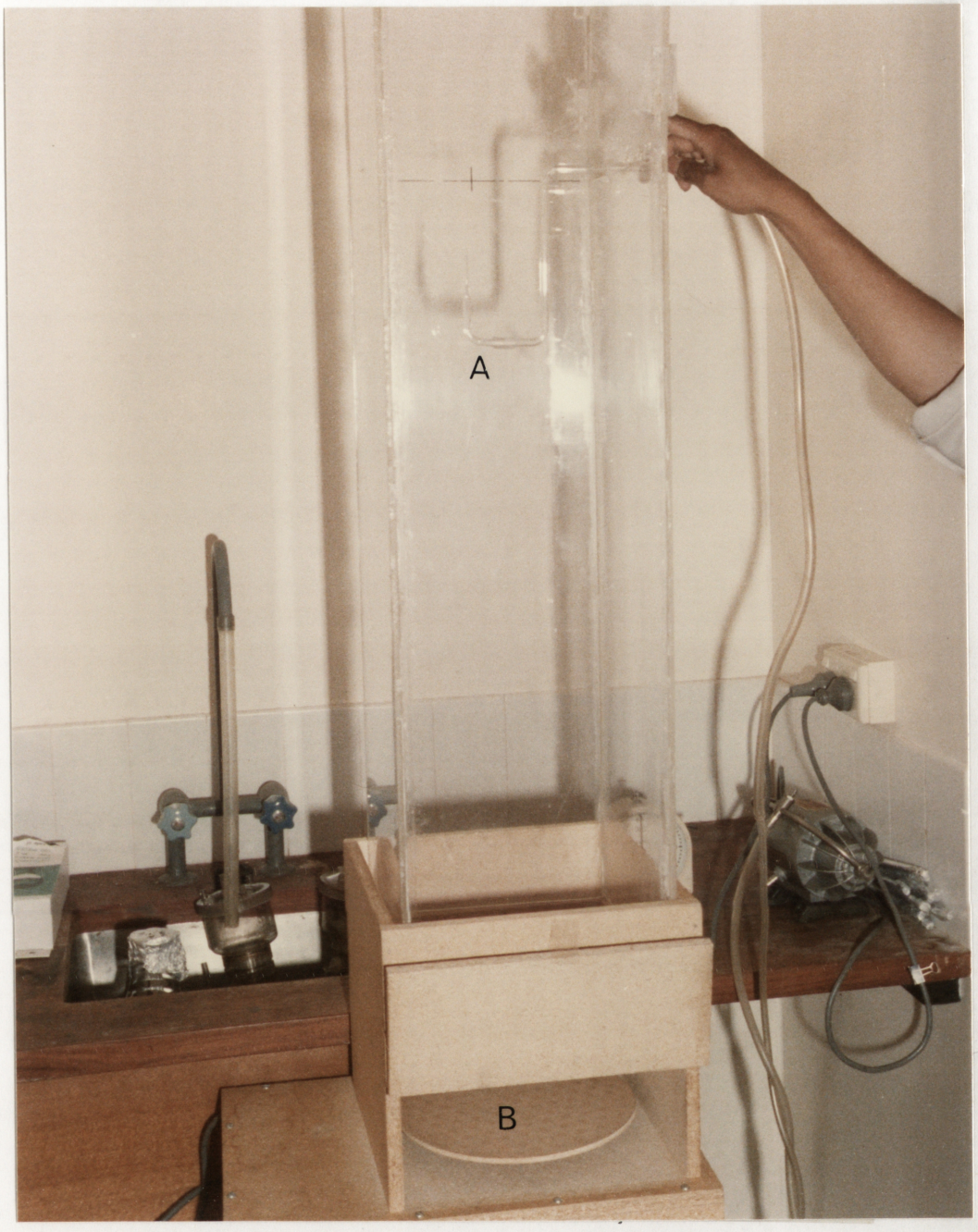


Plate 2.2 The spore settling tower employed to inoculate leaf disks of poplar cultivars. A - spore gun. B - revolving base.

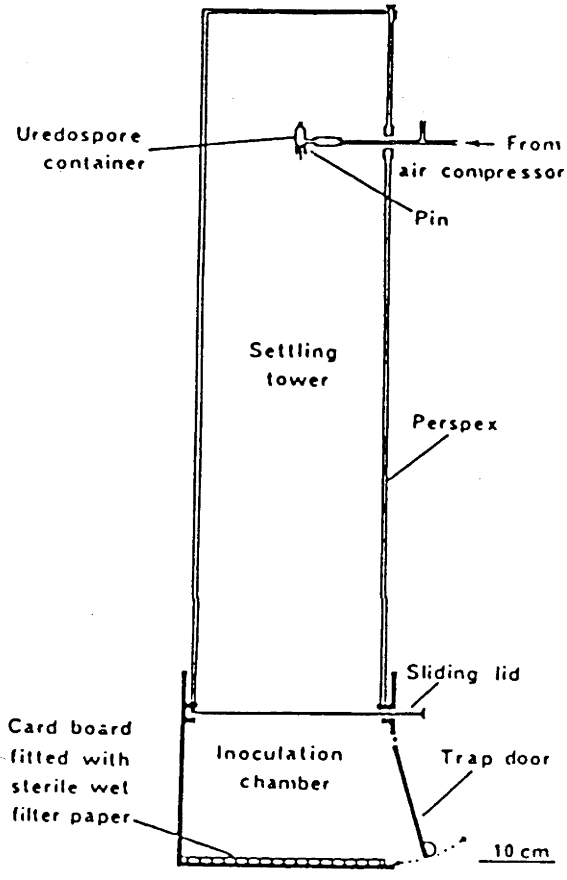


Figure 2.1 Diagram of the spore settling tower employed to inoculate leaf disks of poplar with urediniospores of M. medusae (Reproduced from Sharma et al, 1980).

washed with 70% ethanol, cleaned with sterile blotters and blown dry, and when not in use, it was maintained in a sterile room lit with UV lamps. Preliminary studies using P. x euramericana cv. I-488, demonstrated that this method prevented inter-racial contamination of the urediniospores in successive inoculations. The method was satisfactory for routine assesment of disease phenotype, due to the uniform deposition of the spores [variation in the deposition of the spores within and between incoulations non significant ($P > 0.05$)]. Occasionally, when this criterion of uniformity was not met, inoculation was repeated on separate sets of leaf disks. Uniformity in urediniospore deposition is essential in precise comparisons of disease traits, particularly those measuring disease severity. The. levels of appropriate replication, inoculum dosage and timing of exposure to the inoculum were adopted following preliminary studies (Chandrashekar, 1981; Omar, 1978; Rayner, 1983; Singh, 1983).

2.5 INCUBATION OF LEAVES AND LEAF DISKS

2.5.1 INCUBATION OF LEAVES : Inoculated leaves used for production of urediniospores, were floated on gibberellic acid (section 2.4.1) in plastic Petri dishes (10 cm) and incubated in control growth cabinets (Plate 2.1b), at '16±1 °C, 100±10 $\mu Em^{-2}s^{-1}$, 16 h photoperiod', referred to hereafter as 'standard environment'. The gibberellic acid solution was replenished usually after 10 days. To produce the source inoculum for all studies, the races were multiplied on P. x euramericana cv. I-488, a universal suscept to all races of M. medusae (Singh, 1983).

Because of the demonstrated environmental sensitivity of this pathosystem (Singh and Heather, 1982 b and c, and 1983), this 'standard environment' was used throughout the study (unless indicated otherwise) in the multiplication of the urediniospores and to assess the disease phenotype in sections I and III. This 'standard environment' is suitable for uniform and maximal disease expression, thus enabling comparative examination of the related experiments in the study. Day (1974) has detailed the need for use of optimum uniform environment in genetic studies of host-pathogen relationship.

2.5.2 INCUBATION OF LEAF DISKS : Inoculated leaf disks were placed on plastic foam, which was thoroughly soaked with GA, located in divided Petri dishes (Plate 2.1f) and incubated initially for 24 h in diffused light (30-50 $\mu Em^{-2}s^{-1}$, 16±1 °C, 16 h photoperiod). These conditions ensured uniform, maximal germination and subsequent penetration of leaf disks by germ tubes (Singh and Heather, 1982c; Prakash, unpublished). Subsequently, the Petri dishes were incubated in 'standard environment' in controlled growth cabinets (Plate 2.1b) (L. B. Phytotron, CSIRO) (Morse and Evans, 1962) lit with cool flourscent tubes (Philips®, Australia) (Plate 2.1). The positions of the Petri dishes in the chamber were random and changed every two days for first eight days of incubation. The temperature readings were taken daily (recorded on a thermograph and a thermometer) and light intensity was recorded using a LI-700® Photometer (LI-COR, USA), which was calibrated periodically.

2.6 OBSERVATIONS ON DISEASE EXPRESSION/PHENOTYPE

2.6.1 QUALITATIVE ASSESSMENT : The qualitative observations on disease phenotype were recorded at the end of the disease monocycle (time from inoculation until the apparent cessation of uredinial production and when majority of the uredinia show signs of disintegration) (Fig. 2.2) on an Infection Type (IT) scale, 0 - 4, of increasing disease severity (Plate 2.3), viz.,

0 - immune; no macroscopic symptoms
1 - resistant; necrotic and/or chlorotic flecks only
2 - moderately susceptible; few and or small uredinia, sometimes surrounded by necrotic flecks
3 - susceptible; many medium sized uredinia or few large sized uredinia
4 - highly susceptible; large number of uredinia showing extensive sporulation

Sometimes, in addition, an IT of 1a was adopted to indicate a resistant reaction (IT - 1) accompanied by very few (two to three) minute uredinia per leaf disk, while 'b' indicates relatively larger uredinia within the

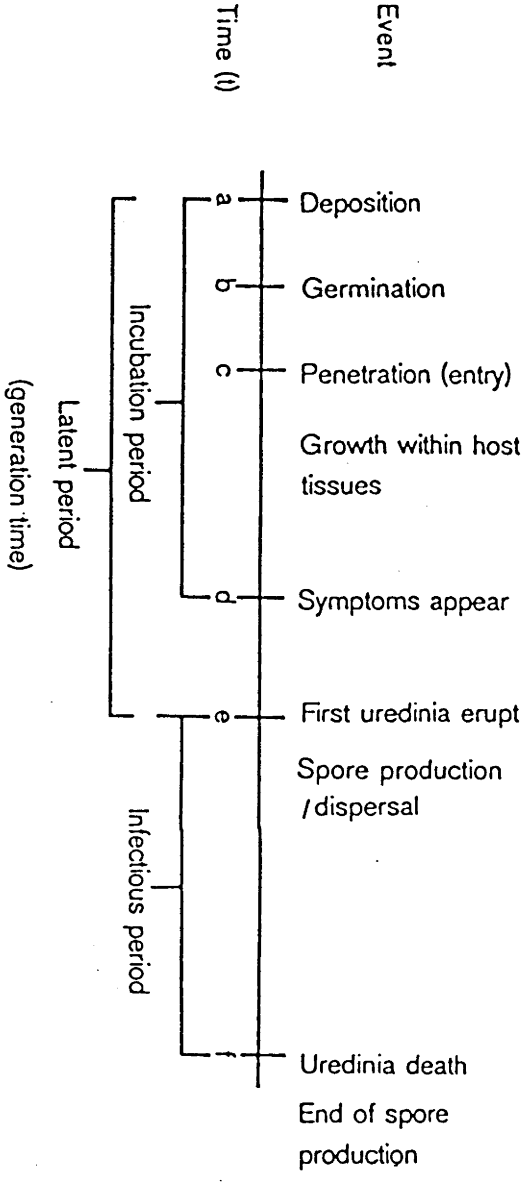


Figure 2.2 Diagramatic representation of a typical monocyle of a rust (Reproduced from Teng and Close, 1978).

IT class. An addition of + sign to an IT value indicates the presence of numerous necrotic spots.

An IT of 0 - 1 was considered resistant/avirulent indicating incompatibility (-), while an IT 2 - 4 was classed susceptible/virulent indicating compatibility (+), reaction. The IT 2-4 scale, in a limited sense, also indicates an increasing aggressiveness in terms of disease severity of the isolate of the pathogen or a decreasing resistance in the cultivar of the host. Virulence Spectrum indicates the number of cultivars, within a group of differential cultivars, on which a race or isolate of the pathogen was recorded as virulent (IT, 2-4).

2.6.2 QUANTITATIVE ASSESSMENT : In a typical monocycle (Fig. 2.2), several measurements of disease expression can be made. These can be broadly classified into 1) those denoting timing of disease expression or rate of disease progression 2) those indicating severity or amount of disease produced. In this thesis, terms on components of the monocycle adopted are as described by Teng and Close (1978). The disease phenotype is a product of interaction of host and pathogen genotypes (Flor, 1956). Thus, to maintain relevance in the discussion, in cases dealing with the variability in the pathogen on a specific host, the variation in disease phenotype is referred to as aggressiveness, and in those dealing with variability in host with specific pathogen race, it is referred to as a component of resistance. Importance of some of these traits of aggressiveness in quantitative aspects of host-pathogen relations and in epidemiology, has been detailed (e.g. Johnson and Taylor, 1976; Zadoks and Schein, 1979; Teng and Close, 1978; Parlevliet, 1979; Kranz, 1983). The term 'aggressiveness' used in the study is the quantitative measurement of the various traits of disease expression on a specific cultivar or cultivars and does not necessarily imply lack of specificity as suggested by Vanderplank (1968) or Robinson (1976). For reasons of brevity, abbreviations of these traits and other parameters are used throughout the thesis. The following general quantitative parameters have been adopted, but the choice of specific parameters varies between studies and has been specified in each chapter :

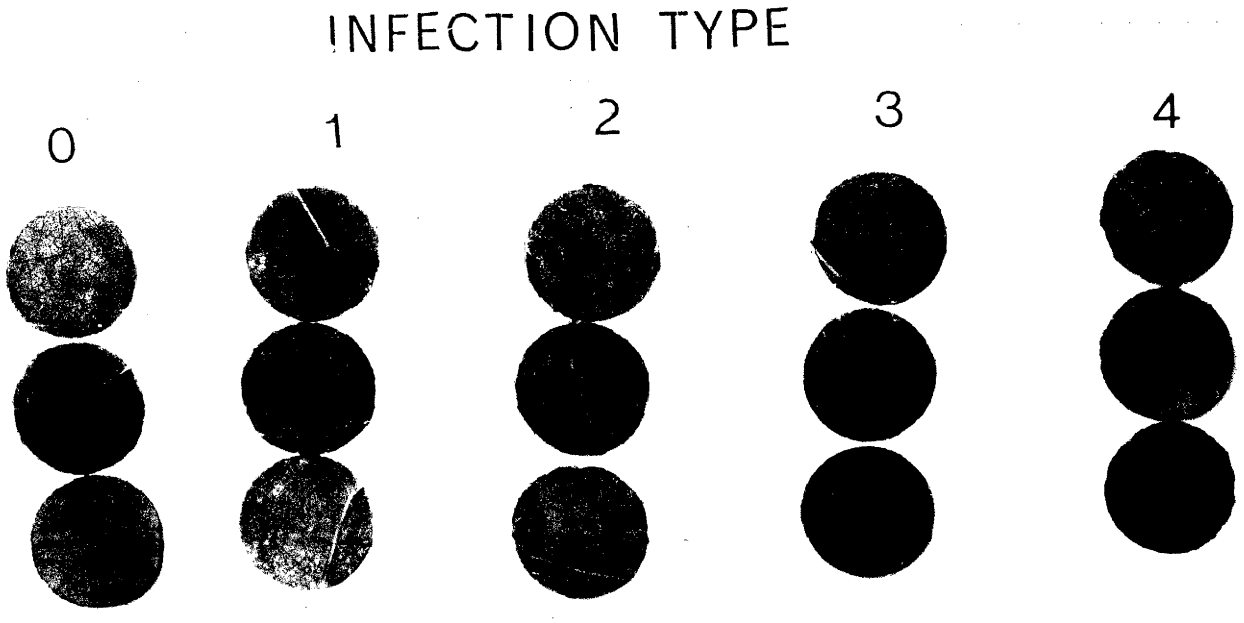


Plate 2.3 Leaf disks showing representative Infection Type (IT) levels on a 0 - 4 scale of increasing disease severity.

2.6.2.1 INCUBATION PERIOD (DAYS) TO FLECKING (IPF) : Time between inoculation (ta) and appearance of flecks (td) (Fig. 2.2); flecks are localised chlorotic or necrotic areas, the earliest visible symptoms of disease. IPF was recorded on individual leaf disks in a particular treatment and the mean computed.

2.6.2.2 LATENT PERIOD (DAYS) TO PRODUCTION OF THE FIRST UREDINIUM (LP1) : Time from inoculation (ta) to the appearance of the first well developed uredinium (te) (Fig. 2. 2); usually many uredinia erupt together on this day. Mean LP1 was computed from observations of individual replicate leaf disks in a particular treatment; an important trait which reflects the rate of disease progression (Parlevliet, 1979).

2.6.2.3 LATENT PERIOD (DAYS) TO PRODUCTION OF 50 PER CENT UREDINIA (LP50) : Time from inoculation (ta) to the production of 50 per cent uredinia (between te and tf in Fig. 2.2) recorded at the end of the monocycle. Number of uredinia produced per leaf disk recorded daily and LP50 calculated for each disk and mean LP50 computed.

2.6.2.4 UREDINIA PRODUCED PER LEAF DISK (ULD) : Number of uredinia was recorded at the end of the monocycle on individual replicate leaf disks and mean ULD computed. As fixed inoculum load was used within a study, ULD reflects the infection or disease efficiency and thus again it is an important trait (Nelson, 1979). The cumulative total uredinia was employed because observations in the middle of the monocycle might not correctly indicate the relative susceptibility/ aggressiveness of the host-pathogen complex (Vanderplank, 1978).

2.6.2.5. UREDINIA PRODUCED PER DAY (UPD) : Number of uredinia produced at the end of monocycle (ULD) was divided by the length of the monocycle (in days) for each replicate leaf disk, and mean UPD computed. This trait reflects the rate of disease progression and parallels the 'rate of infection' **r** detailed by Vanderplank (1963, 1968).

2.6.2.6 UREDINIOSPORES PRODUCED PER SQUARE MILLIMETRE OF LEAF AREA (USM) : This trait combines the interaction of infection efficiency (ULD) and the fecundity of the pathogen on a specific host (USU), and is a

cumulative value which may depend on the age of the pustules. The estimation of USM and USU is described in section 2.6.2.8.

2.6.2.7 UREDINIOSPORES PRODUCED PER UREDINIUM (USU) : This trait reflects the fecundity of the pathogen on a specific host in a given environment. It is also a cumultive value; although a very useful trait, it needs careful interpretation as it has been observed to be negatively correlated with uredinial density (Prakash, unpublished), and thus precise comparision of USU among treatments is more valid when the ULD of the treatments do not differ widely.

While IPF, LP1 and LP50 indicate the timing of disease expression or rate of disease progression, ULD, USM and USU denote the extent of disease severity or amount of disease produced.

2.6.2.8 ESTIMATION OF USM AND USU : These two traits were measured at the end of the monocycle, after final ULD has been recorded. Two batches of five leaf disks randomly selected from a particular treatment were transferred into sterile McCartney bottles containing 5 ml of sterile 0.1% agar solution (w/v) (Difco Agar). This solution contained Tween-20® (Polyoxy ethylene sorbitan monolaurate, ICA American Inc., Willington, USA), a wetting agent, at the rate of 3-4 drops per litre. The urediniospores were dislodged into the solution by agitating the bottle vigorously on a mechanical shaker (wrist-action type shake, c. 120/sec) at 16_{+1}°C. Spore counts from this suspension were made using a haemocytometer (Neubauer Counting Chamber) and six random counts per bottle were made (each count a mean of four squares), giving twelve replications per treatment in all studies except in chapter 14 where only five leaf disks were used (five replications). The number of urediniospores in the suspension was estimated (Sheridan, 1976) by using the following formulae:

$$\text{Number of spores/ml} = \frac{\text{Number of spores in 4 corner squares}}{\text{4 (= number of squares)}} \times 10\,000$$

2.6.2.8.1 ESTIMATION OF USM : From the above equation (section 2.6.2.7) the number of urediniospores produced on five leaf disks (= number of

urediniospores in 5 ml solution) was calculated and USM computed as follows:

$$USM = \frac{\text{Number of spores in 5 ml solution}}{5 \times 170 \text{ (= leaf area in mm2 used in preparing 5 ml solution)}}$$

Mean USM was calculated from the replicate bottles for each treatment.

2.6.2.8.2 ESTIMATION OF USU : The USU was computed per bottle as

$$USU = \frac{\text{Number of spores in 5 ml solution}}{\text{Number of corresponding uredinia}}$$

The mean USU was calculated for replications within a treatment.

2.7 STATISTICAL ANALYSIS OF DATA

The quantitative data of disease expression were analysed with suitable statistical tools, using a computer package, GENSTAT (General Statistical Program) (Alvey et al, 1982) on a UNIVAC 1100/82 computer, Computer Services Center, The Australian National University. The progeny frequency distribution was tested for goodness of fit to a normal distribution, and skewness and kurtosis was computed, on the MPL (Maximum Likelihood Program) computer package (Ross, 1980) with UNIVAC computer (Chapter 14). The graphic presentation of some of the data were made on the LISA APPLE computer, while a few figures were drawn by hand; thus the variations in style of presentation of the figures.

2.7.1 ANALYSIS OF VARIANCE : Analysis of Variance (ANOVA) was used to partition the variation due to the treatment components and their orders of interaction. The basic assumption of ANOVA, normality of the distribution of errors and the homogeneity of variance (homoscedasticity) (Neter and Wasserman, 1974) were tested by examining the residual scatter plots (with a MACRO sub-package of GENSTAT) and if

deviant, data were subjected to suitable transformations (usually square root or $\log_e$ transformations) to suit the above requirements. The transformed data reduces the errors due to scaling.

In the ANOVA involving fixed model experiments, when the interaction term (s) were significant, interaction degrees of freedom and sum of squares were added respectively to the residual degrees of freedom and sum of squares, and the variance ratio for the major factors were recalculated. This adjustment permitted the discussion of the significance of individual major factors despite the significance of the interaction terms (Chandrashekar and Heather, 1981; R. Cunningham, pers. commun.). The details of ANOVA in the thesis, are presented as mean sum of squares (MSS) in some chapters, while in other instances, variance ratio (VR) is also presented.

2.7.2. TESTS OF SIGNIFICANCE : The variance ratios in the ANOVA were tested for their significance by F test, while the significance of differences between treatment cell means (multiple comparison) were tested by a Least Significant Difference (LSD) test, using the value of standard errors of difference from the appropriate interaction component (Steel and Torrie, 1960), and two-tailed student's 't' value with a probability level of P=0.05 (except in Chapter 10).

2.7.3 DISEASE PROGRESS CURVES : The individual regression curves of disease progression were constructed (using untransformed values) from daily observations of uredinial numbers per leaf disk (usually commenced two days after the date of eruption of first uredinium) over the length of the monocycle. These curves were fitted (Sharma et al , 1980) employing the following model:

$$y_i = b_0 + b_1 x_i + b_2 x_i^2 + e_i$$

Here, y_i is the fitted disease level on day x_i, and the best fit was decided on the maximal coefficient of determination obtained. Such comparisons eliminate the possibility that differences in disease severity between any two treatment means are an artefact of the time at which the disease was assessed.

2.7.4 THE REGRESSION ANALYSIS : The regression analysis was performed by least squares method using GENSTAT language and the relationship between any two variables was tested by deviation method of the regression analysis (Neter and Wasserman, 1974) which involves fitting models beginning with a linear model and testing the significance of deviation with higher models, and the model chosen as appropriate was that which would best explain the variation in data.

Other additional statistical techniques adopted are detailed in the pertinent chapters.

SECTION 1

" these mutable and treacherous creatures"
– a plant breeder's comments on fungi

SECTION I

VARIABILITY AND SELECTION IN MELAMPSORA MEDUSAE FOR VIRULENCE AND AGGRESSIVENESS ON POPLAR, AND A STUDY OF THE RELATIONSHIP BETWEEN THE TWO PARAMETERS

- A SUMMARY OF BACKGROUND LITERATURE

CHAPTER 3

INTRODUCTION

Variability in plant pathogens has been recognised since early this century (Eriksson, 1894; Barrus, 1911). Stakman and Piemeisel (1917) clearly established the concept of physiologic races in Puccinia graminis Pers. tritici Erikss. and Henn. of wheat, i.e., forms within a pathogen species which are morphologically alike but which differ in their virulence reaction within a set of host cultivars. Subsequently, such variability for qualitative host specificity was recognised in many plant pathogens, and detailed genetic studies have been made in some instances; see reviews by Flor, 1956 and 1971; Hooker and Saxena, 1971; Day, 1974; Webster, 1974; Burnett, 1975; and McIntosh and Watson, 1982.

Co-evolution of the host and its pathogen has been postulated in those systems which follow a gene-for-gene relationship, where each gene for resistance in the host interacts with a corresponding gene for virulence or avirulence in the pathogen (Mode, 1958; Flor, 1971; Leonard, 1977). Consequently attempts to control plant diseases by the use of resistant host genotypes can result in frequent, and some times spectacular, shifts in the racial composition of the population of plant pathogens i.e. the specificity in the host-pathogen relationship results in selection pressure by the host genotype on the mixture of biotypes making up the population of the pathogen. In crop plants, Johnson

(1961) has referred to this as 'man guided evolution of the plant pathogens'. Although, in certain host genotypes resistance has remained stable over time, the economic consequence of the loss of effectiveness of resistance genes in some crops due to the shifts in pathogen populations, are well known (Vanderplank, 1968; Watson, 1970; Marshall, 1977).

Thus, an understanding of the aspects of changes in pathogen behaviour, including the mechanisms by which pathogens generate variability for virulence and aggressiveness, the effect of increased virulence spectra on the aggressiveness and fitness of the isolates, and the host selection patterns for increased aggressiveness on the pathogen, may assist in identifying those strategies of disease management through host resistance that are likely to be stable. In a perennial crop like poplar, where genetic manipulation of the host is laborious and time consuming, rendering the identification of specific genes for resistance difficult, precise studies on genetics of pathogen can be rewarding in studying indirectly the host behaviour for disease resistance. Kinloch (1982) commented 'more rapid progress in gene identification might be made by shifting the focus of genetic analysis from hosts to pathogens, which can be genetically manipulated with much lower investment in time, space and cost'.

A review of some of the studies and concepts on genetic basis of variability in fungi, genetic control of virulence and aggressiveness, the gene-for-gene theory, the relationship between virulence spectrum and the aggressiveness, and on coevolution of the host pathogen system has been provided separately in Appendix 4. Here only the relevant points have been summarised.

Variation is essential for survival to the fungi in a changing environment. Mutation is the ultimate basis of most observed genetic variation. Both spontaneous and induced mutants for virulence have been reported in other rust fungi, although the literature on changes from virulence to avirulence is lacking. Forms of asexual mechanisms generating variability, e.g. heterokaryosis, mitotic recombination etc. are known also to contribute to the variability in fungi. In some well studied pathogens, avirulence has usually been observed to be controlled

by single dominant genes, often interacting with dominant resistance genes in the host, on a gene-for-gene basis. There are limited reports on the genetic control of aggressiveness in plant pathogens, and on the role of this factor in the evolution of the pathogen. However, differences in aggressiveness traits within and between races have been noticed in many plant pathogens, and in some rusts, races or biotypes exhibiting cultivar specificity for aggressiveness traits have been observed. Some plant pathogens have been observed to be more aggressive on the cultivar on which they were isolated suggesting a degree of adaptation by the pathogen on those cultivars. Some studies have shown that races with a broader virulence spectrum are less aggressive on certain cultivars, while certain studies have shown the reverese. There are fewer studies on the microevolution of the pathogen in various host environments. Most authors agree that, in an association of host and pathogen, both influence each other thus resulting in coevolution. In their theoretical models, some authors have shown that when hosts and pathogens interact on a gene-for-gene basis, stability can be maintained. However, in other models, few authors have shown, that non-specific or additive interaction between hosts and pathogens can result in stability. For details and references on these points, the reader is referred to the Appendix 4.

CHAPTER 4

RESPONSE TO GAMMA IRRADIATION, INDUCED AND NATURAL VIRULENT MUTATIONS IN *MELAMPSORA MEDUSAE*

4.1 INTRODUCTION

Mutations are the mainstay of genetic research. Since Muller (1927) showed that mutations can be artificially induced by using X rays, detailed analysis of the hereditary behaviour in organisms have been conducted through mutagenesis and, particularly in microorganisms, much of our knowledge in genetics is derived from artificially induced mutants.

In plant pathogens, mutation to virulence has been observed to occur spontaneously (Watson, 1957; Flor, 1958; Day, 1974) and has also been induced artificially using irradiation (UV, X rays, gamma rays and neutrons) and certain chemicals (EMS and NTG) (Keit and Boone, 1954; Baker and Teo, 1966; Teo and Baker, 1975; Simons, 1979). Many workers have advocated the use of a mutational approach in genetic studies of plant pathogens (Day, 1968, 1974; Day *et al*, 1983; Rowell *et al*, 1963; Teo and Baker, 1975; Simons, 1979). However, Day (1979) has commented that, mutants in plant pathogens have had surprisingly little effect in furthering our understanding of the pathosystem.

The major problem with artificially induced mutation is the risk of producing potentially destructive new races and the possible escape of these into the field, thus defeating the very purpose of the study (Day, 1974; Kwon and Oh, 1977; Simons, 1979). However, with a careful approach and stringent precautions, and when conducted under controlled, artificial conditions, induced mutation to virulence has a vast potential to contribute to our understanding of genetic behaviour of pathogens (Kwon and Oh, 1977), and possibly is less risky when compared to studies with genetically engineered plant pathogens.

Flor (1958 and 1960) suggested that mutation to virulence in the pathogen is as important as mutation to resistance in the host.

Some authors have suggested that, if new races of pathogens could be produced experimentally before they occur in nature, they could, in theory, be used to screen host populations in advance of the natural occurrence of such races (Kwon, 1974).

Also, an association between the rates of natural mutation and induced mutation for virulence has been observed in some rusts (Flor, 1958). Thus, knowledge of the relative mutation rates of specific genes for virulence could be useful in choosing those resistance genes in the host against which mutation to virulence in the pathogen occurs at lowest frequency (Brock, in Kwon and Oh, 1977; Luig, 1978a); hence helping to discern the stable and labile loci in the pathogen (Zadoks, in Kwon and Oh, 1977).

In Australia, Mc Intosh (in Kwon and Oh, 1977) was able to supplement, by induced mutagenesis, the combinations of virulence genes available in culture collection, thus improving the efficiency in selection of broad spectrum resistance in wheat to P. graminis f.sp. tritici. A similar strategy was adopted by Notteghem (1977) in rice blast disease. Parlevliet (1983b) has stressed the necessity for use of individual pure races of pathogen with broad virulence spectrum, as opposed to mixtures of races, to identify broad spectrum host resistance,. An important problem with using hypersensitive type resistance to control diseases, is the inability to determine the degree of susceptibility a cultivar will have when a compatible race of the pathogen arises (Clifford, 1974). According to Parlevliet (1983a), ideally the cultivar should have many major genes embedded in a partially resistant or slow rusting background. Induced mutations can help to assess, under carefully controlled conditions, the residual effect of background resistance of such cultivars carrying 'defeated' major genes.

Apart from practical uses, induced mutation can contribute to our fundamental knowledge of plant pathogens, and of their relationship with the host. For example, Schwinghamer (1959), from studies on induced mutation to virulence in Melampsora lini (Pers.) Lev. by **X** radiation, by observing the kinetics of dose-frequency curves, suggested that mutation resulted from deletion of chromosomal segments carrying

avirulence genes. This information led Pryor (1977) to suggest that virulence results from loss of function in the pathogen and thus, emphasised the importance of 'cost of virulence' in pathogen, which has obvious implications in evolution of the pathosystem (Leonard, 1977). Similarly, Ellingboe (1978), suggested the positive recognition of avirulence alleles in an incompatibility system from studies on temperature-sensitive mutants (Gabriel et al, 1979); this has signficant evolutionary and epidemiological implications (Parlevliet, 1983a). Flor (1960), using two of the virulent mutations induced by Schwinghamer, demonstrated the linkage of Ap, Ap2 and Ap3 loci in M. lini.

Rowell et al (1963) used the mutational approach to derive a virulent (isoline) from an avirulent line of P. graminis f. sp. tritici; this enabled them to construct, and demonstrate for the first time, a 'quadratic check', which has been much used to understand the host-pathogen relationship (Martin and Ellingboe, 1976; Ellingboe, 1977, Sidhu, 1975). Luig (1978b) by treatment with Ethyl Methane Sulphonate (EMS), a chemical mutagen, demonstrated the linkage of two avirulence genes in P. graminis f. sp. tritici. Mutations have also been induced to obtain colour markers, which facilitate race mixture studies (Brown, 1975; Falahati-Rastegar et al, 1983). From a practical view point, induced mutation to virulence has a potential use in the control of plant weeds using pathogens with broad virulence spectrum and high aggressiveness (Leonard, 1982). Induced mutation for avirulence has potential applications in induced resistance or cross protection of the plants against potentially virulent races of the pathogen (Kuc, 1983). The mutational approach has also been employed to produce fungicide insensitive biotypes of the pathogens, facilitating studies on stability of various fungicides (Fry et al, 1984; Joseph and Coffey, 1984).

The current work on artificial induction of virulent mutation in M. medusae was undertaken, because of the proposed relevance of the mutation as a predominant source of variability in this pathogen at Australia (Appendix 4), to estimate the frequency of induced mutation rate, and for possible use of the mutant isolates (as near isolines of the wild type avirulent race thus minimising the effect of other background genes; Leonard, 1977) in further epidemiological studies, and

in the study of inheritance and selection of broad-spectrum host resistance (Section III).

Mutation studies employing gamma irradiation necessitates preliminary studies on radiosensitivity of the organism, to estimate lethal doses and to fix dosage limits for subsequent routine mutation work (Schwinghamer, 1958). Although spore germination has been adopted as a criterion to assess such survivability in certain other fungi (Kwon and Oh, 1977), prior studies with M. medusae showed that urediniospore germination was not a suitable criterion to predict infection survival in this fungus (Appendix 5).

This chapter reports the radiosensitivity of three races, 1A, 4A and 5A, of M. medusae to increasing levels of gamma irradiation, (assesed by incubation period to flecking and uredinial survival; Expt. 4.1), radiation induced mutation frequency of race 5A (Expt. 4.2), and occurrence of spontaneous mutation for virulence on P. deltoides cv. T-173 (Expt. 4.2) in race 4A of M. medusae .

4.2 MATERIALS AND METHODS

The races employed in the study are virulent on P. x euramericana cv. I-488 (a universal suscept) on which they produce numerous uredinia with copious sporulation but they are avirulent on P. deltoides cv.T-173.

4.2.1 SPORE MULTIPLICATION AND IRRADIATION : Cultures derived from single urediniospores of M. medusae, races 1A, 4A, and 5A were used in the study assessing sensitivity to gamma irradiation (Expt. 4.1) and of races 4A and 5A in the study of mutation rate (Expt. 4.2). Race 1A was not available for use in the latter study due to insufficient inoculum. Urediniospores were produced in vitro under controlled conditions of artificial light and temperature, on detached leaves of P. x euramericana cv. I-488 (section 2.5.1.; Chapter 2). For gamma irradiation, 30 mg of freshly harvested urediniospores (c. 80 % spore water content), of each race sample contained in separate glass vials were rotated on a turntable during exposure to a Co60 source, 4.8 Grays

(Gy) per minute, for specific periods, at room temperature, to obtain 100, 200, 400, 600, and 1000 Gy (10 Gy = 1 kR) of exposure. Spore water has been shown to affect radiosensitivity and mutation frequency in some other fungi, including M. lini and P. graminis f. sp. tritici (Schwinghamer, 1958 and 1959). Non-irradiated spores served as a control. Subsequently the spores were dried in vacuo over silica gel (12 h) and P_2O_5 (12 h) to reduce the spore water content to c. 20%. The drying of spores reduces clumping in subsequent deposition in the spore settling tower.

4.2.2 INOCULATION : Leaf disks of cv. I-488 were used to assess the radiosensitivity of the three races (Expt. 4.1), and to assess the survivability (control) in the mutation rate study (Expt. 4.2), while cv. T-173 was used to assess the mutation rate (Expt. 4.2). Five mg of dried urediniospores of each isolate from each irradiation dose was deposited on separate batches of leaf disks in a spore settling tower (Section 2.5.2). In the mutation experiment, an 8 mg spore load was used for the 600 and 1000 Gy doses as a partial compensation for the expected reduced survival of urediniospores at these levels of irradiation (Schwinghamer, 1959 and Appendix 5). Subsequent to inoculation, leaf disks were supported on plastic foam in closed Petri dishes containing 10 mg/L gibberellic acid (GA), and incubated at 'standard environment' (16±1°C, 100 uEm-2s-1 light intensity and 16 h photoperiod). Twenty replicate leaf disks of cv. I-488 were used for each race/dose treatment in Expt.4.1, while in Expt. 4.2, 10 replicate leaf disks of cv. I-488 and 20 of cv. T-173 per race/dose treatment were inoculated simultaneously.

4.2.3 OBSERVATIONS : Incubation period (in days) to flecking (IPF) on cv. I-488 was recorded, and the mean (20 replicates) calculated for each treatment in Expt. 1. Uredinia per leaf disk (ULD) produced on cv. I-488 were counted at the termination of the experiment (14-20 days) as an indicator of relative infection potential and uredinial survival percent (SP) was computed as,

$$SP = \frac{\text{Mean ULD for the dose}}{\text{Mean ULD for control (0 Gy)}} \times 100$$

Mutation frequency (MF) in Expt. 2 was computed for race 5A as,

$$MF = \frac{\text{Mean ULD on cv. T-173 for the dose}}{\text{Mean ULD on cv. I-488 for the dose}} \times 100$$

As a precaution, the study was conducted completely in vitro, and at the conclusion of the experiment, the leaf disks of cvs I-488 and T-173 (after isolation of mutant uredinia) were steam sterilised to avoid possible release of treated spores into the field.

4.2.4 STATISTICAL ANALYSIS : Data were tested for homoscedasticity and normality of error variance (section 2.7.1.). The analysis of variance (6 doses X 3 races), regression analysis between irradiation levels and survival, estimation of correlation coefficients between IPF and ULD were conducted with GENSTAT computer package (Alvey et al 1982; Section 2.7). Prior to analysis, the data for ULD were square root transformed to satisfy the above requirements.

4.3 RESULTS

4.3.1 SENSITIVITY TO GAMMA IRRADIATION (EXPT. 4.1) Incubation period to flecking (IPF) of the three races on cv. I-488 generally increased linearly with increasing levels of irradiation from 0 to 1000 Gy but the response of race 1A to irradiation beyond 200 Gy had a quadratic component (Fig. 4.1). The race, dose, and race x dose interaction were significant ($P < 0.001$) in explaining the variation in IPF, however races were the major determinant of such variation (Table 4.1)

While, irradiation dose, race and the race x dose interaction were highly significant ($P < 0.001$) determinants of ULD, irradiation dose was the most important contributor to such variation (Table 4.1). The number of uredinia produced by all the races decreased rapidly with increased irradiation dose, but such reduction was more pronounced in race 1A compared to races 4A and 5A (Fig. 4.2). Races were also compared for their sensitivity to radiation by employing regression analysis to fit curves to the relationship of survival percentage (y)

Table 4.1 Analysis of variances[a] for incubation period to flecking (IPF) and uredinia produced per leaf disk (ULD)[b] for three races of M. medusae on cv. I-488 and six doses of gamma irradiation.

Source	D F	IPF		ULD[b]	
		MSS[c]	VR[d]	MSS	VR
Race	2	117.38	173.10	46.55	74.33
Dose	5	86.24	127.18	152.20	2432.01
Race X Dose	10	14.84	21.88	26.33	42.04
Residual	342	0.67	-	0.63	-
Total	359	2.98	-	3.71	-

[a] All the variances are highly significant ($P < 0.001$).
[b] Using square root transformed data.
[c] Mean Sum of Squares.
[d] Variance Ratio.

Table 4.2 Relationship of survival percentage and doses of Co^{60} irradiation for three races of M. medusae and correlation coefficients between incubation period to flecking (IPF) and uredina produced per leaf disk (ULD; transformed) across all levels of irradiation.

Race	Regression relationship[b]	Nature of curve	Coefficient of Determination[a]	Correlation between IPF and ULD
1A	$\log_e y = 4.605 - 0.0582x + 0.0002x^2$ (0.0094) (0.0001)	Quadratic	0.942	- 0.820
4A	$y = 100 - 1.445\ x + 0.0066x^2$ (0.1875) (0.0021)	Quadratic	0.940	- 0.501
5A	$y = 100 - 0.7393x$ (0.0707)	Linear	0.877	- 0.593

y = percentage survival expressed as percentage of control, x = dose of gamma rays in Gy.

[a] Coefficient of determination (R^2) adjusted to degrees of freedom; explains the percentage of variance accounted for in the model.

[b] Standard errors are given in paranthesis for coefficients of x and x^2

Figure 4.1 Incubation period to flecking (IPF) of three races of M. medusae on P. x euramericana cv. I-488, over increasing levels of gamma irradiation.

Figure 4.2 Uredinia produced per leaf disk by three races of M. medusae on P. x euramericana cv. I-488, over increasing levels of gamma irradiation.

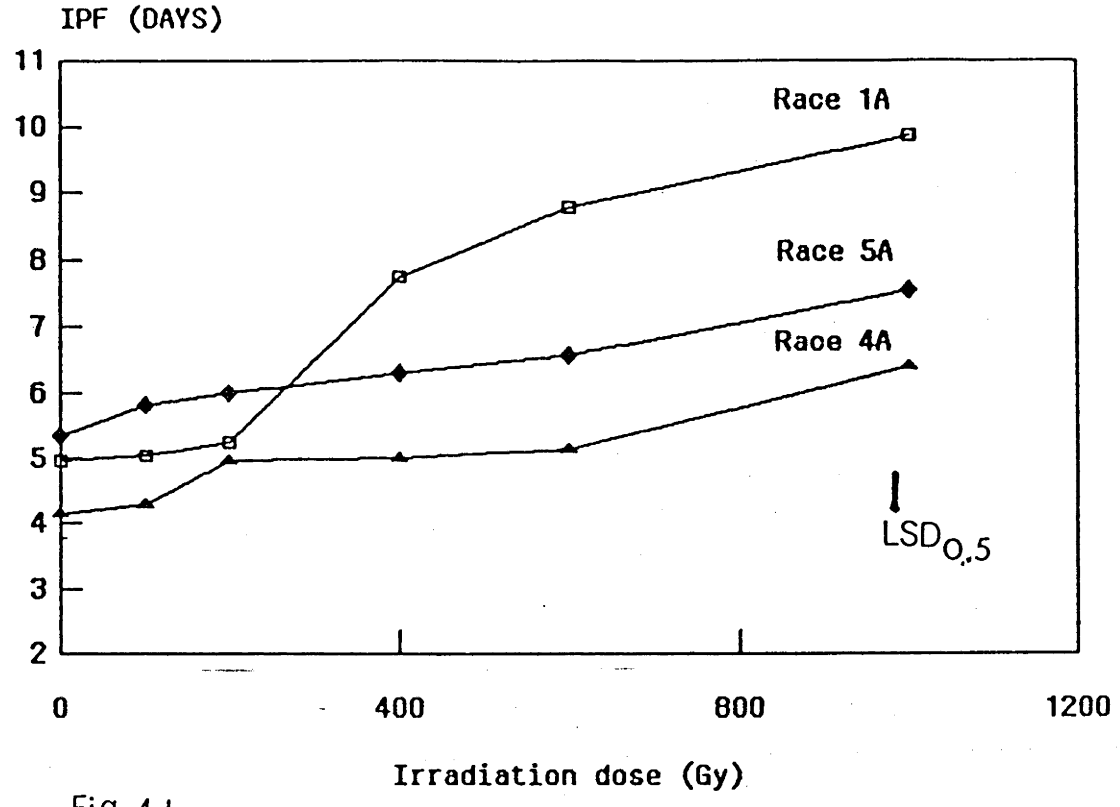

IPF (DAYS)
11
10
9
8
7
6
5
4
3
2
Race 1A
Race 5A
Raoe 4A
LSD0.5
0
400
800
1200
Irradiation dose (Gy)

Fig. 4.1

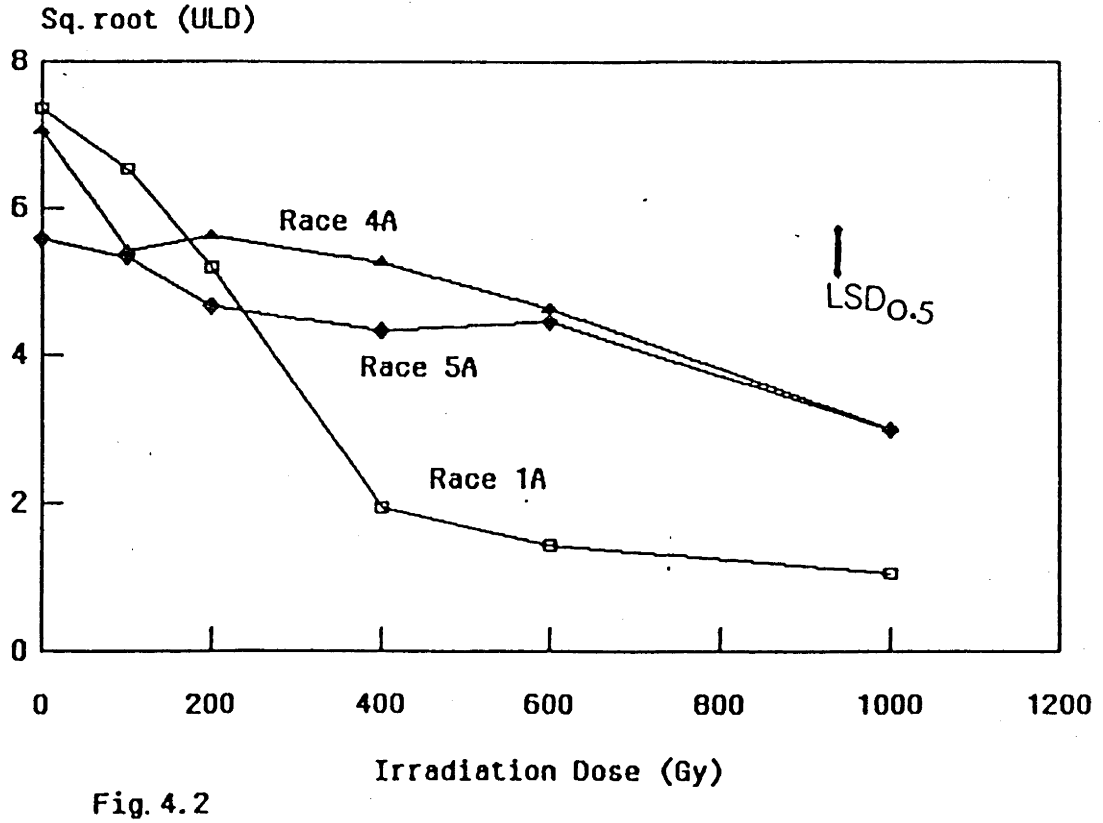
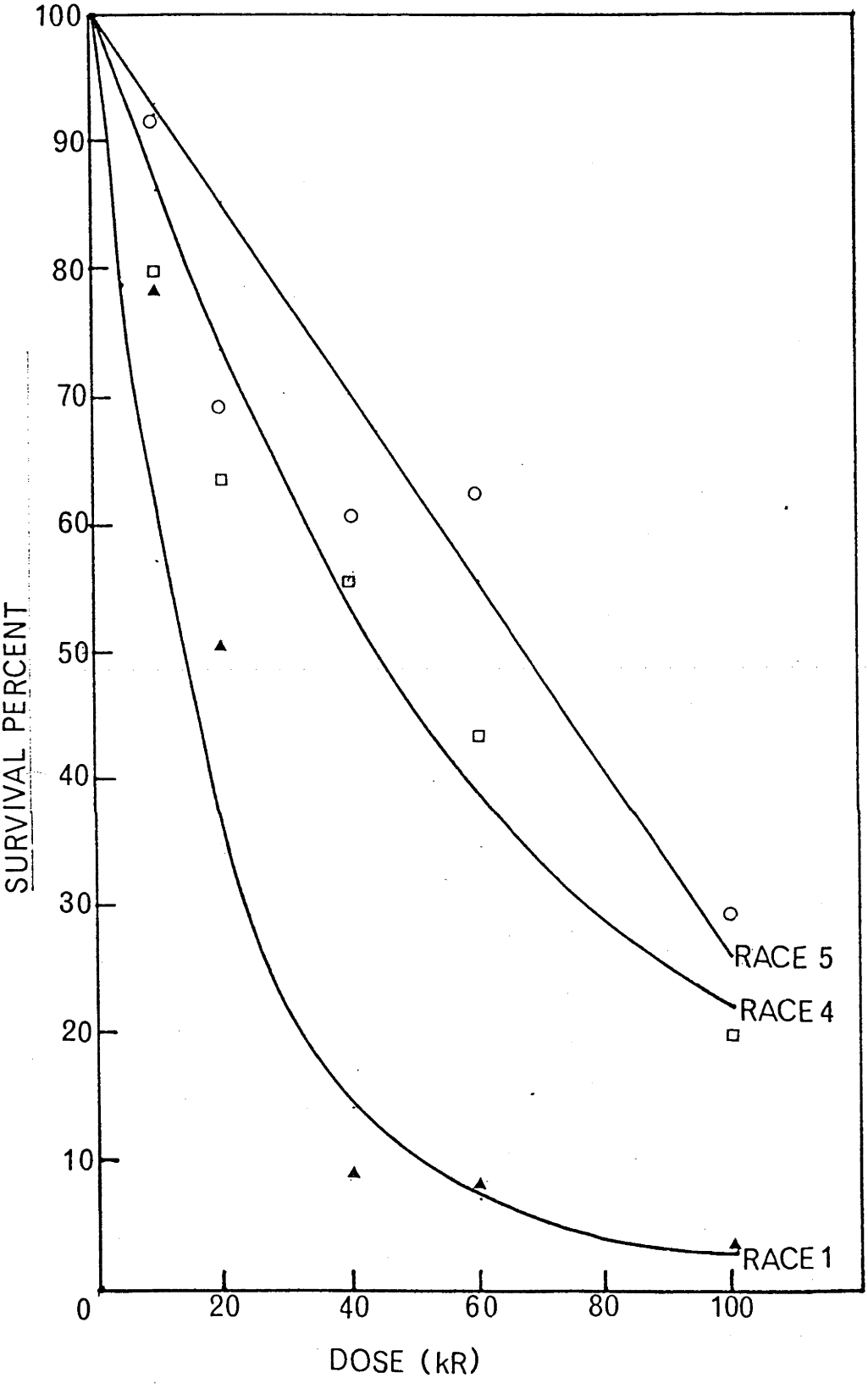

Sq. root (ULD)
8
6
4
2
0
Race 4A
Race 5A
Race 1A
LSD0.5
0
200
400
600
800
1000
1200
Irradiation Dose (Gy)

Fig. 4.2

Figure 4.3 Uredinial survival* of three races of M. medusae on P. x euramericana cv. I-488, over increasing levels of gamma irradiation.

*(as percentage of control)


100
90
80
70
60
50
40
30
20
10
0
SURVIVAL PERCENT
20
40
60
80
100
DOSE (kR)
RACE 5
RACE 4
RACE 1

(basis ULD on cv. I-488) and irradiation level (x) (Fig. 4.2). A quadratic model of the form $\log_e y = b_0 - b_1x_1 + b_2x_1^2$ produced the highest coefficient of determination for race 1A (Table 4.2). Because of pronounced reduction in survivability at 400 Gy, the Log_e model illustrated the response of this race better than with the untransformed y. For race 4A, a similar model, but without log transformation, was the most appropriate. The addition of the quadratic component did not significantly increase the coefficient of determination ($P > 0.05$) for race 5A, hence a linear model of the type $y = b_0 - b_1x_1$ was employed. Since for each race ULD for the control was taken as 100%, all the curves were forced through the origin and b_1 taken as 100 (4.506 for race 1A, as $\log_e 100$) [Fig. 4.2, Table 4.2].

For all levels of irradiation employed, the order of increasing sensitivity (basis, uredinial survival) in the races was 5A > 4A > 1A. Correlation coefficients between IPF and ULD for the three races (across all levels of irradiation, Table 4.2) were negative and highly significant ($P < 0.01$). Although the correlation between IPF and ULD was higher in race 1A (- 0.82) than race 4A (-0.50) or 5A (-0.59), correlation coefficients were greater at higher, than at lower, levels of irradiation (details not presented).

4.3.2 MUTATION FREQUENCY (EXPT. 4.2)

On cv. T-173 leaf disks inoculated with untreated (control) urediniospores of race 5A, did not produce any uredinia indicating the absence of any contamination or spontaneous mutation in this collection, but untreated spores of race 4A produced a few uredinia on the leaf disks of this cultivar. Thus for calculating mutation frequencies, data for race 4A were omitted as the control samples of this race possibly contained spontaneous mutants virulent on cv. T-173. For race 5A, mutation frequency on cv. T-173, computed as percentage of suvivors, increased linearly from 0.89% at 100 Gy to a maximum of 1.68% at 400 Gy, decreased from 400 to 600 Gy and remained relatively constant from 600 Gy to 1000 Gy (Fig. 4.4).

The latent period for the mutants was long compared with that

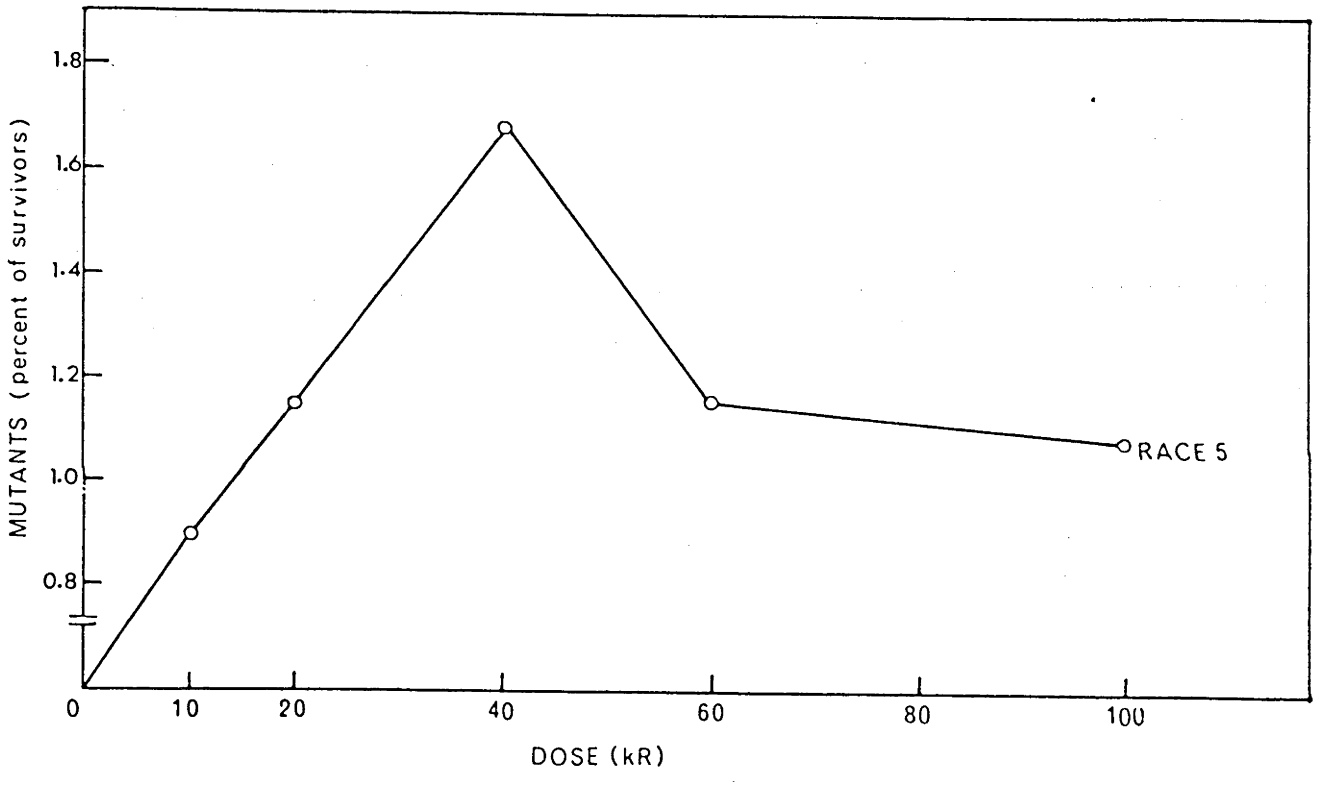


Figure 4.4 Virulent mutation frequency of race 5A of M. medusae on cv. T-173 over increasing levels of gamma irradiation.

of race 5A and final counts of ULD were taken 3-4 days later than survival counts. However, when cv. T-173 was inoculated again with the progeny of the mutants, development of uredinia was relatively more rapid and its aggressiveness was higher than the nascent mutants.

4.4 DISCUSSION

4.4.1 SENSITIVITY TO GAMMA IRRADIATION (EXPT. 4.1) : The three races of M. medusae differ in radiosensitivity as assessed by incubation period to flecking and uredinial survival.

Although IPF increased linearly with increasing levels of irradiation this response was most obvious in race 1A and least evident in race 4A (Fig. 4.1) while in all the races the increase was pronounced beyond 600 Gy. Possibly, irradiation of the spores slows down those metabolic activities significant in colonisation of the host and thus the period of establishment of the fungus was increased. Although both race and dose were significant factors contributing to the variation in IPF, the contribution of the race to such variability was greater than that due to the dose, indicating the inherent difference among these three races in their response to the irradiation.

The curves relating survival to the level of gamma irradiation, were quadratic for races 1A and 4A, but linear for 5A (Fig. 4.3, Table 4.2). Over the whole of their range (100-1000 Gy) the ranking of the races for survival (sensu uredinial production) was 1A < 4A < 5A. Radiosensitivity is influenced mainly by ploidy, nuclear volume, the stage of development of the cell when exposed and environmental conditions (Davies and Evans 1966, Conger et al 1982). However, these three races are dikaryotic (functionally diploid), have similar spore size and received identical environmental treatments and thus differences in radiosensitivity, are rather unexpected. Any deleterious change in the basic compatibility genes in M. medusae, a biotroph, would result in lethality i.e. analogous to an auxotroph on a unsupplemented medium. The differences in radiosensitivity of the races to irradiation may be due to their variation in the composition of basic compatbility genes (Ellingboe, 1978; Gabriel et al, 1979; Parlevliet, 1979 and 1983a). In the present study, the ranking of races for

aggressiveness (basis ULD at 0 Gy; Fig 4.2) 1A > 4A > 5A, parallels that for radiosensitivity at all levels of irradiation (Fig. 4.3). This suggests that the alleles for enhanced aggressiveness may be considerably more sensitive to irradiation damage. In Aspergillus flavus (Padwal-Desai et al, 1976), toxigenic strains were found to be more radioresistant than non-toxigenic strains, while among rusts, some species (e.g. Puccinia coronata Corda f. sp. avenae Fraser and Led.) have been observed to be more resistant than others (e.g. M. lini) to irradiation (Schwinghamer 1958).

For all the three races the correlation coefficient ('r') between IPF and ULD across different levels of irradiation (Table 4.2) were negative and highly significant ($P < 0.01$).

4.4.2 MUTATION FREQUENCY (EXPT. 4.2) : The frequency of mutations virulent on cv. T-173, computed as percent of survivors on cv. I-488, was higher than that obtained for M. lini in a similar study using gamma rays (Schwinghamer 1959). The increase in mutation rate with the rise in dose from 100 to 400 Gy, is in agreement with the results of Schwinghamer (1959). However, the decrease in the mutation rate above 400 Gy possibly results from the small sample size, insufficient compensation in the spore load at 600 and 1000 Gy to allow for the expected reduction in survival of the urediniospores or from clumping of spores during inoculation of the leaf disks at higher (8 mg), compared to normal (5 mg), spore load. A field collection of M. medusae has shown virulence on cv. T-173 (Singh and Heather 1982a); however, in previous studies, races 4A and 5A, produced incompatible reactions on cv. T-173 (Prakash, unpublished). When used in the present project, race 4A produced occasional uredinia on this cultivar and also, despite subsequent attempts to purify the race by leaf replica technique (Appendix 3) and single spore isolation, such natural virulent mutants arose frequently in this race. In the methods employed in this laboratory, contamination is unlikely and hence a high frequency of natural mutation during the multiplication of race 4A on cv. I-488 is the most likely explanation of this change in virulence. Such high frequencies of mutation in a race, suggest that the race may be heterozygous for avirulence, or the locus may be highly mutable, and

also suggest a possible activity of transposing elements (Appendix 4). Similar observations on high frequency of certain virulence genes have been noticed (Ou and Ayad, 1968; Bartos *et al*, 1969). Flor (1958) observed that natural mutants in *M. lini* were not qualitatively different from those induced artificially and concluded that genes most responsive to mutagenic effects of irradiation may be the least stable under natural conditions.

Though all the virulent mutants initially exhibited slow growth on cv. T-173, their progeny showed relatively higher aggressiveness when used to re-inoculate this cultivar. Thus the delayed IPF and longer latent period seen initially, could be due to non-genetic effects of irradiation. The high frequency of mutation, greater than usually observed (Schwinghamer, 1959), suggests that genes for avirulence in race 5A on cv. T-173 may be dominant and heterozygous. In the heterozygous condition the loss of the avirulence gene immediately results in a virulent phenotype (Schwinghamer, 1959). Schwinghamer (1959), by observing 'two-hit' kinetics, concluded that mutation to virulence could result from the deletion of a chromosomal segment carrying the avirulent gene. However, in this study the mutation frequency response was linear (0-400 Gy) rather than quadratic and hence the virulence may have resulted from single-hit point, mutation or terminal deletion.

Currently under field conditions in Australia, cv. T-173 appears resistant and thus is being used as a source of leaf rust resistance gene/s (R. R. Willing , *pers. commun.*). The resistance to race 5A in cv. T-173 appears to be controlled by a single dominant gene (Section III). Caution should be exercised in wide and continous deployment of such major resistance genes from this cultivar as this study suggests that mutants, virulent to this cultivar, can arise rapidly. However, the epidemiological importance of mutants, or variants with an increased spectrum of virulence, rests in their ability to colonise and reproduce in competition with the existing wild types on various cultivars and under differing environmental conditions (Day, 1979).

The immune or hypersensitive type resistance in cv. T-173 is broken by the mutant isolates, whether natural or artificial. A qualitative gene-for-gene relationship between races of *M. medusae* and

cultivars of poplars is the simplest genetic basis of such interactions (Flor, 1956, Parlevliet and Zadoks, 1977). It would be valuable to know if the change in virulence toward cv. T-173 involved the same loci in both irradiated and natural mutants and also to determine if such changes in virulence involved simultaneous changes in a/virulence towards other cultivars. Similarly, it is of epidemiological importance to test the aggressiveness and competitive abilities of these mutant biotypes in relation to those of the parental wild type on the susceptible cultivar on which they arose. These aspects are the subjects of subsequent chapters.

4.5 SUMMARY

Three races of M. medusae differ in sensitivity, as assessed by incubation period to flecking and uredinial survivability, to increasing levels of Co60 gamma irradiation. In race 5A, the mutation to virulence on P. deltoides cv. T-173 was induced by irradiation, and the frequency of mutation was maximum (1.688%) at 400 Gy. Further, a high frequency of natural occurrence in race 4A of mutants virulent on this cultivar was observed. The results are consistent with a possible gene-for-gene basis of the relationship between major resistant genes in poplar and certain virulence genes in M. medusae.

CHAPTER 5

VIRULENCE SPECTRUM OF RADIATION INDUCED AND NATURAL VIRULENT MUTANTS OF *MELAMPSORA MEDUSAE* ON CULTIVARS OF POPLAR

5.1 INTRODUCTION

The artificially induced mutants of race 5A and natural mutants of race 4A were selected for their virulence on cv. T-173 . A comparison of the irradiated and natural mutants for their virulence on a range of cultivars would indicate if, with mutation for virulence in these isolates on cv. T-173, the virulence spectrum had been significantly altered on other cultivars. Apart from the differences in radiation induced and natural mutants, such a study would be expected to reveal, i) the possible association or disassociation of virulences on other cultivars due to linkage, and ii) the changes in relative aggressiveness of these isolates on those cultivars on which the both the wild type and mutants are virulent. Information on linkage or the pleiotropic nature of these virulence alleles would suggest which cultivars share common resistance genes. Alexander *et al* (1984b) have discussed the implication of such virulence associations in deployment strategies of resistance genes.

This chapter reports the results of two separate studies conducted to determine the virulence spectrum, on a range of *Populus* cultivars, of radiation induced (Expt. 5.1), and natural, virulent mutants (Expt. 5.2) of races 5A and 4A, respectively, in comparison with the wild types from which they were derived.

5.2 MATERIALS AND METHODS

5.2.1 THE PATHOGEN ISOLATES: Induction of the mutants of race 5A of *M. medusae* virulent on T-173, cultivar resistant to race 5A, has been detailed (Chapter 4). A total of 36 uredinia, developed on leaf disks

of cv. T-173 following inoculation with urediniospores of race 5A, gamma irradiated at one of five [100, 200, 400, 600 & 1000 Gray (Gy)] (1 kR=10 Gy) dosage levels. Spores collected from five randomly selected, mutant uredinia were multiplied separately under controlled conditions on detached leaves of cv. T-173 (2 generations) and subsequently on cv. I-488 (4 generations) to obtain 5 mg of dry urediniospores of each mono-uredinial isolate, referred to hereafter as mutant isolates 5M1, 5M2, 5M3, 5M4 and 5M5 (Expt. 5.1). These mutant isolates had been isolated from uredinia which developed from spores exposed to 200, 600, 100, 100 and 100 Gy irradiation, respectively.

As race 4A had developed spontaneous or natural virulent mutants, this race was suspected to be a composite mixture of biotypes virulent and avirulent on cv. T-173. A leaf replica technique (see appendix 3 for details) developed by the author, effectively separated these biotypes. Isolate 4B was isolated for its avirulence on cv. T-173 and is thus considered a wild type (although, subsequently it exhibited slight virulence on this cultivar; Chapter 6, Table 6.7), while isolates 4M1 and 4M2 were isolated (randomly from many such mutants) for their virulence on cv. T-173, and were multiplied as described for radiation induced mutants (Expt. 5.2).

5.2.2. THE HOST CULTIVARS: Eleven cultivars, representing 4 species of poplar (*P. deltoides* cvs PC/75 series comprising 40-2, 10-3, 7-2, and 7-4; cvs 60/164, W-79/304, W-79/307; *P.* x *euramericana* cvs I-154 and 65/70; *P. alba* L. cv. Hickeliana and *P. nigra* L. cv. Italica), were used in Expt. 5.1, to assess the reaction of radiation induced virulent mutants, while ten cultivars (*P. deltoides* cvs 79/307, 60/164, PC/75 series comprising cvs 7-2, 7-4, 40-2 and 10-3; *P.* x *euramericana* cv. I-154; *P. alba* cv. Hickeliana; *P. simonii* cv. Simonii; *P.nigra* cv. Italica) were used in Expt. 5.2. Both studies were conducted separately and the differences in the series of cultivars employed in the experiments resulted from the unavailability of leaves of comparable maturity in certain cultivars.

5.2.3 INOCULATION AND INCUBATION: In both experiments, freshly harvested, dried urediniospores (5 mg) of each of the isolates were

deposited separately on randomised leaf disks (five replicates) of each cultivar and coverglasses (1.32 cm2), placed on the base of a spore settling tower (section 2.4.2). The uniformity of deposition (less than 5% variation between inoculations), and germination (more than 94% in all six isolates), of urediniospores were checked on the coverglasses. Following inoculation, the leaf disks were placed on plastic foam soaked with gibberrelic acid, sealed in glass Petri dishes and incubated in growth cabinets at the 'standard environment' (section 2.5.1).

5.2.4 OBSERVATIONS AND ANALYSIS: At the end of the monocycle (section 2.6.1) which ranged from 14 days to 26 days depending on the cultivar/isolate combination, leaf disks were scored for Infection Type (IT) on a 0 - 4 scale (section 2.6.1). An isolate was classified as avirulent (IT 0-1) or virulent (IT 2-4). In this study, the aggressiveness of an isolate on a specific cultivar was measured as mean uredinial number per leaf disk (ULD), at the culmination of the monocycle (section 2.6.2.4). Though most cultivars used in the study are susceptible to some races of M. medusae (Singh and Heather, 1982a and b), as a precautionary measure, leaf disks were steam sterilized before they were discarded.

The values for ULD were transformed (square root) to ensure homoscedasticity and normality of error variances (section 2.7.1) and subjected to analysis of variance (5 or 6 isolates X 11 cultivars) with the inclusion and exclusion of data for race 5A in separate analysis, for Expt. 5.1. Student's 't' test, with standard errors of differences of means of the interaction component, was employed to compare mean (square root) ULD for all cultivar/isolate combinations. Similarly, in Expt. 5.2, analysis of variance was performed (3 isolates X 10 cultivars) with transformed ULD values before comparison of mean values.

5.3 RESULTS

5.3.1 RADIATION INDUCED MUTANTS (EXPERIMENT 5.1) : On the eleven cultivars, the virulence spectra of all five radiation induced mutant isolates was wider (7-9 out of 11 cultivars) than that of wild type race

Table 5.1 Infection types[a] of race 5A and its five radiation induced mutants of *M. medusae*, on eleven cultivars of *Populus* spp.

Isolates	Dose (Gy)	HOST CULTIVARS											Virulence Spectrum[b]
		79/307	79/304	60/164	7-4	7-2	40-2	10-3	I-154	65/70	Italica	Hickeliana	
Race 5A	0	2+	1a+	1+	1+	4	0	1	2+	2	4	0	5
5M1	200	3	2	3	3	2	1	1	4	3	2	2	9
5M2	600	4	3	3	3	1	1	1	4	4	2	1[a]	7
5M3	100	4	2	3	3	1[a]	1[a]	1	4	4	3	2	8
5M4	100	4	2	3	3	1	1	2	4	4	3	2	9
5M5	100	4	2	3	3	1	1[a]	1	4	4	3	1[a]	7

+ Necrotic flecks were recorded also on these combinations (mesothetic response).

[a] on a 0 - 4 scale, resistant to susceptible. (IT 1a indicates presence of 1 or 2 minute uredinia).

[b] Number of cultivars on which the isolate demonstrates virulence i.e. IT 2 - 4.

Table 5.2 Analysis of variance for uredinial number per leaf disk (ULD; sqaure root transformed) for race 5A and its five radiation induced mutants of M. medusae, on eleven cultivars of Populus spp[a].

Source	(a) WITH RACE 5A			(b) WITHOUT RACE 5A		
	D F	MSS	VR	D F	MSS	VR
Isolate	5	18.89	36.983	4	1.93	3.439
Cultivar	10	117.08	229.174	10	129.11	230.027
Isolate x Cultvr	50	11.09	22.172	40	2.44	4.355
Residual	264	0.51	-	220	0.56	-
Total	329	5.97	-	274	5.58	-

[a] All the variance ratios are highly significant ($P < 0.001$)

Table 5.3 Mean number of uredinia per leaf disk (ULD; square root transformed) of race 5A and its five radiation induced virulent mutant isolates of *M. medusae*, on eleven cultivars of *Populus* spp[ab].

	HOST CULTIVARS											
Isolates	79/307	79/304	60/164	7-4	7-2	40-2	10-3	I-154	65/70	Italica	Hickeliana	Mean ULD
5A	2.78[a+]	0.79[a+]	0.00[a]	1.00[a+]	5.81[c]	0.00[a]	0.00[a]	1.08[a+]	1.96[a]	7.73[c]	0.00[a]	1.926[a]
5M1	4.71[b]	2.34[b]	5.36[bc]	4.74[b]	2.46[b]	0.00[a]	0.00[a]	5.17[b]	4.57[b]	3.10[a]	1.66[b]	3.103[b]
5M2	5.77[c]	3.99[c]	6.09[bcd]	5.93[c]	0.00[a]	0.00[a]	0.00[a]	5.34[b]	6.40[d]	3.60[a]	0.56[a]	3.428[c]
5M3	5.58[c]	2.54[b]	4.94[b]	4.83[b]	0.40[a]	0.76[b]	0.00[a]	5.78[b]	5.50[c]	4.82[b]	1.95[b]	3.375[c]
5M4	5.73[c]	2.45[b]	6.24[d]	4.55[b]	0.00[a]	0.00[a]	1.54[b]	5.87[b]	5.23[bc]	4.55[b]	2.10[b]	3.500[c]
5M5	6.09[c]	1.52[a]	4.87[b]	4.32[b]	0.00[a]	1.09[b]	0.00[a]	5.66[b]	5.26[bc]	4.55[b]	0.72[a]	3.101[b]

+ Necrotic flecks were recorded also on these combinations (mesothetic response).
[a] Each value is a mean of five replicates.
[b] Within a cultivar column, values sharing the same letter do not differ significantly ($P < 0.05$).

5A (5 out of 11 cultivars), from which they were derived. Compared to race 5A, the five mutant isolates exibited higher IT on eight, a decreased IT on two and a similar IT on one cultivar (Table 5.1). In the analyses of variance, cultivar, isolate and their interaction were significant ($P < 0.001$) causes of variation in ULD, irrespective of whether race 5A was included (Table 5.2a) or excluded (Table 5.2b) from the analyses; in both instances, the cultivar was the major contributor to variation in ULD.

Race 5A was significantly less aggressive than any of the induced mutant isolates on three of the four cultivars on which both the mutant isolates and race 5A were virulent (Table 5.3). However, on cv. Italica, a universal suscept, the wild type race 5A was the most aggressive isolate employed. When averaged across all the cultivars, mutants 5M1 and 5M5 were significantly less aggressive (lower ULD) than the other three mutant isolates. However when the mutants are ranked for relative aggressiveness on individual cultivars, no single isolate is consistently the most aggressive (Table 5.3). For example, though 5M5 was the most aggressive isolate on cv. W-79/307 it was the least aggressive of the mutants on cvs W-79/304 and 7-4. This inconsistency explains the significant isolate/cultivar interaction in the ANOVA (Tables 5.2a and 5.2b).

On this group of cultivars there was no consistent relationship between the dosage of gamma irradiation and relative level of virulence (Table 5.1) or aggressiveness (Table 5.3), in the induced mutants. For example, mutants 5M1 and 5M5 which produced low mean ULD (across all cultivars) were induced at doses 200 and 100 Gy respectively; while significantly higher ULD was produced by 5M2, 5M3 and 5M4 induced at 600, 100 and 100 Gy respectively (Table 3).

5.3.2 NATURAL VIRULENT MUTANTS (EXPERIMENT 5.2) : The virulence spectrum of the isolate 4B was narrower (3 out of 10 cultivars) than that of isolate 4M2 (7 out of 10 cultivars) and 4M1 (9 out of 10 cultivars) (Table 5.4). Both the natural mutant isolates (4M1 and 4M2) could be recognised as separate, distinct races by their qualitative reactions on the ten cultivars (Table 5.4), in contrast to the radiation induced mutants where such differences among the mutants were relatively less

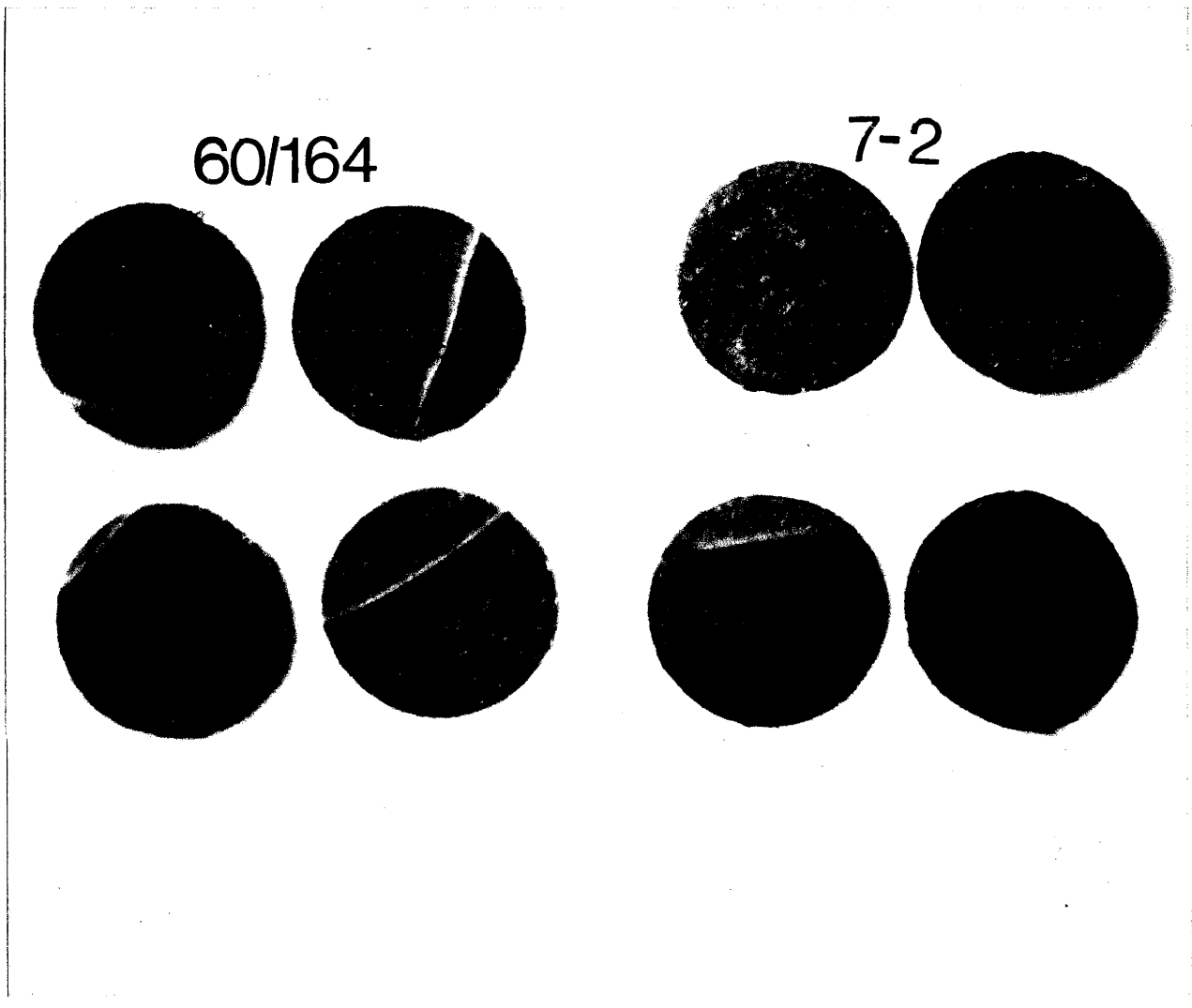


Plate 5.1 The reaction of race 5A (top row) and radiation induced virulent mutant isolate, 5M2 (bottom row), of M. medusae on P. deltoides cultivars 60/164 and 7-2.

Table 5.4 Infection Types[a] (IT) of race 4B and two natural virulent mutant isolates of *M. medusae* on ten cultivars of *Populus* spp.

Isolate	HOST CULTIVARS										Virulence Spectrum[b]
	7-2	7-4	10-3	40-2	60/164	W-79/307	I-154	Hickeliana	Simonii	Italica	
4B	3	0	1	1	0	3	1	0	1	4	3
4M1	2	2	3	1	4	3	4	2	2	3	9
4M2	2	2	1	1	4	3	4	0	2	3	7

[a] Infection type assessed on a 0-4 scale of increasing disease severity (based on number and size of uredinia).

[b] Virulence Spectrum is the number of cultivars on which the biotype is virulent (i.e. IT > 2).

Table 5.5 Analysis of variance of uredinia per leaf disk[a] of race 4B and two natural virulent mutants of M. medusae on ten cultivars of Populus spp.[b]

Source	D F	MSS	VR
Isolate	2	86.17	300.74
Cultivar	9	62.45	192.03
Isolate x Cultivar	18	23.33	67.77
Residual	120	0.32	-
Total	149	8.01	-

[a] using square root-transformed values for ULD
[b] All the values are highly significant ($P < 0.001$).

Table 5.6 Mean number of uredinia per leaf disk (square-root transformed) of race 4B and two natural virulent mutants of *M. medusae* on ten cultivars of *Populus* spp.[a]

	HOST CULTIVARS										
Isolate	7-2	7-4	10-3	40-2	60/164	W-79/307	I-154	Hickeliana	Simonii	Italica	Mean ULD
4B	3.85^{a}	0.0^{b}	0.0^{b}	0.0^{a}	0^{b}	5.57^{a}	0.0^{c}	0^{b}	0.0^{b}	7.47^{a}	1.69
4M1	1.48^{c}	4.67^{a}	3.85^{a}	0.0^{a}	7.31^{a}	4.59^{b}	7.67^{a}	3.73^{a}	4.24^{a}	6.96^{a}	4.45
4M2	2.82^{b}	4.55^{a}	0.0^{b}	0.0^{a}	7.03^{a}	5.13^{ab}	6.41^{b}	0.0^{b}	3.57^{a}	5.03^{b}	3.45

[a] Isolates sharing the same alphabet within a cultivar do not differ significantly ($P > 0.05$)

and were rather continuous. (Table 5.1). Isolate 4B could be distinguished as a race distinct from the isolates 4M1 and 4M2 by its avirulence on 6 cultivars on which either of the latter were virulent (viz., cvs. 7-4, 10-3, 60/164, I-154, Hickeliana and Simonii) (Table 5.4). Isolate 4M1 was virulent on cvs. 10-3 and hickeliana on which isolate 4M2 was avirulent. However the infection types of the isolates 4M1 and 4M2 were similar on those cultivars on which both mutants races were virulent (viz., cvs. 7-4, 60/164, W-79/307, I-154, Simonii and Italica) (Table 5.4). On cv. 40-2, all the three isolates were avirulent.

Although the isolate, cultivar and their interaction were a significant ($P < 0.001$) source of variation in ULD produced by these isolates on ten cultivars, the isolate was the largest contributor to such variation (Table 5.5), which contrasts with Expt. 5.1, where the cultivar was the most important contributor to such variation.

Race 4B was consistently more aggressive (higher ULD) on all the those cultivars on which all the isolates were virulent (cvs. 7-2, W-79/307 and italica) (Table 5.6), while in the previous experiment race 5A was most aggressive only on cv. Italica. However, on the seven cultivars on which only races 4M1 and 4M2 were virulent, the former was relatively more aggressive on five (7-4, 60/164, I-154, Simonii and Italica) and the latter more aggressive on two, cultivars (7-2 and W-79/307) (Table 5.6). Such inconsistencies in ranking for aggressiveness among the races on certain cultivars would explain the significant race x cultivar interaction for ULD observed in the ANOVA.

All the major effects recorded were reproduced when the experiment was repeated employing single isolates of the mutants (5M2 and 4M2), and the races 5A and 4A.

5.4 DISCUSSION

Both artificially induced and natural mutants of M. medusae, when compared to the avirulent wild types from which they were derived, exibited wider virulence spectrum (basis IT) (Tables 5.1 and 5.4) on the

range of cultivars. Thus, based on their reaction on individual cultivars, both radiation and natural mutants groups could be recognised as races (sensu Stakman & Christensen, 1953) distinct from their respective wild types. In contrast the minor differences in IT between individual radiation induced mutant lines on specific cultivars are insufficient to postulate racial differences, although it is possible that if additional cultivars had been included in the study, some of these mutant isolates may have been discerned as distinct races. But the induced mutant isolates could be termed as 'races or pathotypes differing in their quantitative specificity' on these culitvars, because of the differences in aggressivenes (basis ULD) and the significant isolates x cultivar interaction, often accompanied with reversal in ranking (Tables 5.2b, Figure 5.1) (Scott et al, 1980). These results resemble patterns of aggressiveness for isolates of Puccinia hordei Otth. on cultivars of barley (Clifford & Clothier, 1974) and for races of M. larici-populina on cultivars of P.x euramericana (Chandrashekar 1981).

In contrast, the qualitative reaction of the natural mutant isolates 4M1 and 4M2 on this range of cultivars permit their recognition as distinct races (sensu Stakman and Christensen, 1953) (Table 5.3). Similar occurrence of variants differing in virulence within a monospore derived race, has been noticed in other fungi (Ou and Ayad, 1968; Bartos et al, 1969). The isolate 4M1 was avirulent on cvs 10-3 and Hickeliana (IT - 0), while isolate 4M2 was virulent on these cultivars (IT - 2/3). Such discreteness in their reaction of these natural mutant isolates would account for isolate being the largest contributor to variation in ULD in Expt. 5.2, compared to Expt. 5.1 where such differences among isolates were less discrete, and resulted in cultivar being a relatively more significant important to variaton.

Both the mutant groups have been selectively isolated for their virulence on cv. T-173 (Chapter 4). Hence, the higher IT of the irradiated mutants, compared with the wild type race 5A, on cultivars W-79/307 and W-79/304 [progenies of a cross using cv. T-173 (male) and P. deltoides cv. 61/58 (female); R. R. Willing, unpublished], is not unexpected (Table 5.1), as these two cultivars might carry some of the resistance components of cv. T-173. Such an observation can be explained with the interaction model of Parlevliet and Zadoks (1977),

where minor genes in the pathogen (mutant isolates) interact with the corresponding minor genes in the compatible hosts (cvs 79/307 and 79/304) to result in increased aggressiveness and also suggest the influence of background genotype of the host in disease expression as noted by Eskes (1983a). In constrast, the natural mutants 4M1 and 4M2 however, demonstrate decreased aggressiveness when compared to race 4B on cv. 79/307 (Table 5.6), which further demonstrates the differences in specificity of aggressiveness by the races employed in this study.

The differences between cvs 7-2 and 7-4, which are half-sibs from a North American provenance (Eldridge et al, 1973), are more discrete and both cultivars appear to possess mutually exclusive factors for resistance to the wild type and the radiation induced mutants (Table 5.1). Such an observation conforms to the gene-for-gene theory involving major genes (Flor, 1956), and indicates at least the partial disassociaton of the virulence genes as observed in Puccinia graminis f. sp. tritici by Alexander et al (1984b). In Expt. 5.2, although race 4B exibited decreased aggressiveness on cv. 7-2 compared to the virulent mutants 4M1 and 4M2, complete loss of virulence was not observed. Such intermediate virulent types might be similar to those observed in P. graminis f.sp tritici by Watson and Luig (1968a).

The change from avirulence in the wild types, to virulence in all the mutants on cvs I-154, 60/164 and 7-4 (Plate 5.1) suggest that these latter may carry the same resistance gene(s) which was countered by the mutation in the wild types to give virulence on cv. T-173. Alternatively, such an effect could result from the deletion of a chromosomal segment carrying a highly linked cluster of avirulent genes corresponding to the resistance genes in these four cultivars. Schwinghamer (1959) by observing 'two-hit' kinetics for irradiation dose and mutation frequency concluded that mutations to virulence occurred in Melampsora lini as a result of chromosomal deletion. However, for the mutants of race 5A, the relationship of mutation frequency and dose was linear, rather than quadratic, which was indicative of single hit mutation and/or terminal deletion (Chapter 4). Also, as deletions are relatively infrequent in nature (Davies and Evans, 1966) and as such an effect was also observed among natural mutants of race 4A, a change at

single site of the chromosome of the wild type is a distinct possibility.

Such large differences in virulence spectrum between the mutants and the wild types, can still be explained equally by novel genetic mechanisims such as the modification of a super-supressor gene regulating the activity of a cluster of avirulence genes, similar to those observed in yeast (Hawthrone and Mortimer, 1968) and or/due to the activity of site-specific transposable elements acting as a regulatory gene controlling the activity of structural gene complex of these avirulence alleles (Federoff, 1984). Lawrence et al (1981b) have demonstrated the occurence of inhibitory genes controlling the dominant avirulence genes in M. lini, while Tepper and Anderson (1984) have recently suggested that genetic changes involved in evolution of plant pathogen specificity may be at regulatory, rather than structural, loci. Similarly, Watson and Luig (1968a) suggested the role of supressor genes to explain the gradual changes in virulence of P. graminis f. sp. tritici. Also, in M. lini, Lawrence et al (1981a) have observed that virulence genes A-P, A-P1, A-P2 and A-P3 were sufficiently closely linked to belong to the same cistron or were probably allelic, while Flor (1960), using the virulent mutants induced by Schwinghamer (1959), observed that selection for a single mutational event at one locus (A-P) had resulted in concurrent mutational events at two closely linked loci (A-P2 and A-P3). There are many reports of linkage of virulence genes in plant pathogens (Samborski and Dyck, 1968; Luig, 1978b; Statler, 1979; Gustaffson et al, 1983), and this may result in linkage disequilibrium due to the 'hitch hiking or hijacking ' (Thomson, 1977) of certain unncecessary with necessary virulence genes (Wolfe and Schwarzbach, 1978; Leonard and Czochor, 1980; MacKey, 1981; Alexander et al, 1984b). The information on such associaton would have practical utility in planning cultivar mixtures or multilines, particularly those employed to combat asexually reproducing pathogens, where the probability of breaking such linkage disequilibria is lower (Lodge and Leonard, 1984).

The susceptibility of P. alba var. Hickeliana to certain mutants, supports earlier suggestion of mutation advanced to explain the change from resistance to susceptibility of this cultivar in the field

(Sharma and Heather, 1977). In the present study, although the reaction of this cultivar changed from immunity (race 5A) to moderate susceptibility (mutant isolates 5M1, 5M3, 5M4 and 4M2) (Table 5.2 and 5.4), the aggressiveness of the mutants on this cultivar was low (very few uredinia were seen and the latent period was twice the normal) (Figs 5.1 and 5.2). Possibly, as suggested by Parlevliet (1983b) in the barley-leaf rust system, this cultivar might carry a major gene for resistance along with genes for partial resistance or slow-rusting. The mutation to virulence possibly overcame the major genes but the genes controlling quantitative resistance may still have hindered the expression of high aggressiveness.

Four of the five radiation induced mutants were avirulent on cv. 7-2 although race 5A was virulent, and moderately aggressive, on this cultivar (Table 5.1) (Plate 5.1). This loss of virulence among the mutants contrasts with the acquisition of virulence by most mutants on most of the other cultivars. Possibly, the genes for avirulence towards cv 7-2 are recessive and linked to the previously mentioned avirulence complex, as mutations are usually from dominance to recessiveness rather than *vice versa* (Parlevliet, 1983a). Recessiveness of certain avirulence genes in other rusts has been reported (McIntosh and Watson, 1982). Alternatively, such a change to avirulence may result from a mutation leading to loss of function of an inhibitory gene controlling the avirulence reaction towards this cultivar (Lawrence *et al*, 1981b). Similar results were obtained by Griffiths and Carr (1961) in the reaction of UV induced mutants of *Puccinia coronata* f.sp. *avenae* which showed increased virulence on six, similar reaction on three and a complete loss of virulence on one cultivar of oats tested. Luig (1978a) has reported the loss of virulence of certain mutants races of *P. graminis* f. sp. *tritici* upon treatment with a chemical mutagen.

The differences in virulence and aggressiveness among the radiation induced mutants were not related to the irradiation dose in which they were derived. For example, treatments with radiation doses of 100 Gy produced mutants 5M4 and 5M5 which differed in virulence spectrum (9 cvs compared with 7 cvs) (Table 5.1) and in aggressiveness [mean (sq. root) ULD 3.5 compared with 3.1, differring significantly ($P < 0.05$); Table 5.3]. Possibly the differences in aggressiveness result

from random cryptic changes in loci involved with fitness rather than cumulative effects of irradiation, and some of these fitness loci may also be involved in the determination of quantitative specificity observed in these races towards the cultivars.

Despite certain differences, there are similarities between the radiation induced, and the natural, virulent mutants in their reaction with the cultivars common in both experiments. This suggest that both groups of mutants may have undergone changes in the same genetic locus or loci. The general consistency of the reaction of the all the mutants on most cultivars supports this view and suggests that this region of chromosome carrying the avirulence genes may be a 'hot spot' to mutagenesis.

For the irradiated mutant group, virulence (Table 4.1) and aggressiveness (Figure 4.1) on this set of cultivars do not show a consistent relationship, possibly due to lack of major differences in virulence (basis IT; Table 4.1). However, in the natural mutant group, race 4M2 which had the broadest spectrum of virulence was also the most aggressive race (basis, mean ULD; Figure 4.2). This observation contrasts with Vanderplank's (1968) suggestion that virulence (spectra) and aggressiveness would always be negatively correlated. However, on those cultivars on which both mutants and the wild type were virulent, certain mutant lines are relatively more aggressive on particular cultivars (e.g. cv. 79/307; Table 4.3) while they are less aggressive on other cultivars (e.g. cv. Italica; Tables 4.3 and 4.6). It is possible that virulent mutants arising on the former group of cultivars would increase their frequency while on the latter group, they may not. With similar mutation frequencies, number of virulent mutants appearing on susceptible cultivars would be high when compared to that on relatively less susceptible cultivars, due to the larger population of the wild type in the former. For example, in the present study, cv. Italica, which was the most susceptible cultivar to the wild types, is the only universal suscept (sensu Person, 1959), [susceptible to all the known races of M. medusae (Singh, 1983) and also to M. larici-populina (Chandrashekar, 1981)]. On this cultivar, all the mutants (both induced and natural) were relatively less aggressive compared to their avirulent counterparts. Thus virulent mutants appearing on this culitvar would be at relative disadvantage due to their reduced infection frequency

(ULD). Similar observations were made Teo and Baker (1975) on oat stem rust.

Data on comparison of virulent mutants and their parental wild types for several aggressiveness traits on susceptible and resistant cultivars would contribute to the understanding of the epidemiological features of such isolates, and is analysed in the next chapter.

5.5 SUMMARY

When compared to the avirulent wild types, the radiation induced, and natural, virulent mutants of M. medusae, isolated for their virulence towards P. deltoides cv. T-173, exhibited a wider virulence spectrum on a range of Populus cultivars. Although the qualitative reaction of five radiation induced, mutant isolates was similar on the cultivars, the infection types of two natural mutant isolates were sufficiently qualitatively distinct on two cultivars to be recognised as distinct races. Four of the five radiation induced mutant isolates exhibited avirulence on cv. 7-2, on which the wild type race 5A was virulent. There was significant cultivar x isolate interaction, often accompanied with reversal in ranking for ULD, for both the mutant groups on the cultivars. On those culitvars on which both mutants and the wild types were virulent, the wild type was more aggressive (basis ULD) on certain cultivars while the mutants were more aggressive on other cultivars. On some cultivars, there was pronounced similarity of the irradiated and natural mutant groups in their reactions.

CHAPTER 6

RELATIONSHIP BETWEEN INCREASED VIRULENCE SPECTRUM AND THE AGGRESSIVENESS TRAITS IN *MELAMPSORA MEDUSAE*

6.1 INTRODUCTION :

Although many theoretical studies discuss the relative fitness of a virulent mutant allele arising in an avirulent population (Person *et al*, 1976; Leonard, 1977; Leonard and Czochor, 1980; Vanderplank, 1982), there are few precise experimental studies reporting the relative aggressiveness traits of genotypes carrying such a mutant allele, in comparison with the wild type from which they were derived on susceptible and corresponding resistant hosts. Such information has relevance in understanding pathogen evolution, in predicting population shifts in response to host deployment, and in devising disease management strategies (Appendix 4).

Leonard (1977) considers that much of the confusion on the effect of unnecessary virulence genes on fitness of the genotypes has arisen because of the failure to distinguish the effects of individual virulence genes on fitness from the effects of the rest of the genome. To minimise such background effects, he suggests, a) population studies, where changes in gene frequencies are observed in large heterogeneous population of the pathogen in which modifier genes are randomly distibuted between biotypes, or, b) by comparing the near-isogenic lines of the pathogen, differing only at specific locus for virulence. The second approach appears more promising, as it would eliminate the possible interaction of biotypes that may occur in the first method.

Due to the absence of the sexual stage of *M. medusae* in Australia, construction of such isogenic lines is possible only by a mutational approach (cf. Rowell *et al*, 1963). The mutant isolates described in previous chapters, appear ideal for such comparative

studies. Although the results (Chapter 5) suggested that the mutants differ in more than one virulence gene from the wild types, due to the similarities in reaction of the radiation induced and natural virulent mutants on the particular cultivars, it would be reasonable to assume that such changes do reflect natural situations.

This chapter reports the comparative analysis for five aggressiveness traits of mutant isolates (radiation induced, Expt. 6.1 and natural, Expt. 6.2) with the respective wild types, on the susceptible cultivar (I-488) on which they can arise, and on the resistant cultivar (T-173), to which they were selected.

The compatibility (+) or incompatibility (-) of the mutant isolates and the wild types with the cultivars is summarised in Table 1:

Table 6.1: The reaction of cvs I-488 and T-173 to the wild type and the virulent mutants (+ denotes compatibility, - denotes incompatibility)

		THE HOST	
		Cv. T-173 (R)	Cv. I-488 (S)
THE PATHOGEN	Race 5A or 4B1	-	+
	Mutant Isolates	+	+

Due to the ease with which these virulent mutants were obtained, it is possible that avirulence in wild types may be under simple genetic control. The resistance in cv. T-173 to the two wild type races (5A and 4B) appears to be controlled by two separate single dominant genes (Chapter 14).

6.2 MATERIALS AND METHODS

6.2.1 THE PATHOGEN ISOLATES: The methods for production, selection and multiplication of the mutants, (radiation induced and natural) and of the wild types (races 5A and 4B) have been detailed (Chapters 4 and 5). The studies involving radiation induced mutants (Expt. 6.1) and of natural mutants (Expt. 6.2) were conducted separately.

6.2.2 INOCULATION AND INCUBATION: Five mg of freshly harvested, dried urediniospores of each isolate were deposited separately on 15 replicate leaf disks of each cultivar, and on five replicate coverglasses, in a spore settling tower (section 2.4.2; Chapter 2). Uniformity of deposition within, and between, inoculations, and germinative potential (> 95%) was checked on the coverglasses. The inoculated leaf disks were placed on plastic foam soaked with gibberellic acid solution (10mg/L), sealed in 14 cm glass Petri dishes and subequently incubated in controlled growth cabinets in the 'standard environment' (section 2.5.1). The experiments were of factorial design, with 6 isolates X 2 cultivars in Expt. 6.1, and 3 isolates X 2 cultivars in Expt. 6.2.

6.2.3 OBSERVATIONS ON DISEASE SEVERITY AND ANALYSIS : Six aggressiveness traits were studied in Expt. 6.1, viz., incubation period to flecking (IPF), latent period to production of first uredinium (LP1) and 50% uredinia (LP50), uredinia produced per leaf disk (ULD), uredinia produced per disk per day (UPD) and urediniospores produced per square mm of leaf (USM) (see section 2.6.2 for details), and in Expt. 6.2, IPF, LP1, ULD and USM were recorded. Short IPF, LP1 and LP50 and high ULD, UPD and USM are indicative of high aggressiveness of an isolate on a cultivar.

For all the disease parameters, data were tested for the usual statistical prerequisites (section 2.7.1) and appropriately transformed. The data were subjected to analysis of variance, with the inclusion and exclusion of race 5A in separate analysis for Expt. 6.1. For all the isolates on both cultivars (only in Expt. 6.1), individual curves of disease progress (basis ULD), over time (readings commenced at two days from the date of eruption of the first uredinium), were fitted

as described by Sharma et al (1980) using a GENSTAT package (section 2.7.3) employing the following model:

$$y_i = b_0 + b_1 x_i + b_2 x_i^2 + e_i$$

Here, y_i is the fitted disease level on day x_i, and the three groups of curves (5A/I-488, mutants/I-488, and mutants/T-173) were compared pairwise (Sharma et al, 1980) and tested employing an F-test. Mean UPD was computed also for these three groups.

Correlation coefficients between the traits for aggressiveness were calculated using the treatment means of the five mutant lines on two cultivars (Expt. 6.1) and differences between transformed means of the isolates and correlation coefficients were tested for significance employing Student's 't' test (Least significant differences).

The infected leaf disks were steam sterilized before discarding.

6.3. RESULTS

6.3.1. STUDIES WITH RADIATION INDUCED MUTANTS (EXPT. 6.1): Race 5A was completely avirulent on the resistant cultivar T-173. For the traits of aggressiveness, irrespective of the exclusion or inclusion of race 5A (Tables 6.2a and 6.2b) in the analysis of variance, cultivar was most important (Sig, $P < 0.001$) and isolate a lesser but significant ($P < 0.001$) [except for IPF (NS, $P > 0.05$)] determinant of variability while the cultivar x isolate interaction was usually a significant contributor to such variation. When the variance of the interaction components was included in that of the residual, the main effects (except that of isolates for IPF) were still highly significant causes of variation in the traits and thus can be discussed independently despite the significance of the interaction component (section 2.7.1)

The five mutant lines were more aggressive on the susceptible cv. I-488 than on the resistant cv. T-173. (Table 6.3). Although, the ranking of mutants for ULD and USM was relatively consistent on cv. I-488, this ranking was inconsistent between other traits, or within a

Table 6.2 Analysis of variance (mean sum of squares) for six aggressiveness traits [a] of wild type race 5A and five virulent mutant isolates of M. medusae on two cultivars of poplar.

(a) INCLUDING RACE 5A

Source	D F	IPF	LP1	LP50	ULD	UPD	USM
Isolate	5	0.53ns	3.04	12.59	18.76	5.84	27.09
Cultivar	1	86.35	482.49	1042.96	296.73	178.73	109.96
Isolate X Cultvr	5	0.61ns	3.62	3.86	70.66	17.95	42.01
Residual	167	0.54	0.44	0.62	0.82	0.38	0.15[†]
Total	178	1.08	3.53	7.45	4.94	2.03	3.32[†]

[†] Residual and total degrees of freedom for USM is 132 and 143 respectively.

(b) EXCLUDING RACE 5A

Source	D F	IPF	LP1	LP50	ULD	UPD	USM
Mutant	4	0.61ns	2.61	5.21	14.41	5.17	2.82
Cultivar	1	72.51	423.36	879.85	67.92	71.81	13.92
Mutant X Cultvr	4	0.61ns	3.21	3.82*	4.32	1.56	1.35
Residual	140	0.59	0.48	0.66	0.93	0.38	0.16
Total	148	1.08	3.44	6.81	1.84	1.03	0.40

All unmarked values are highly significant, $P < 0.001$; * $P < 0.05$; ns - not significant, $P > 0.05$.

[†] Residual and total degrees of freedom for USM is 110 and 119 respectively.

[a] The six aggressiveness traits were incubation period to flecking (IPF), latent period to production of first uredinium (LP1), latent period to production of 50% uredinia (LP50), number of uredinia per leaf disk (ULD; square root transformed), number of uredinia produced per day (UPD), and number urediniospores produced per square mm of leaf area (USM; Log_e +1 transformed).

Table 6.3 Means[ab] of six aggressiveness traits[c] of the wild type race 5A and five radiation induced virulent mutant isolates of M. medusae on the susceptible cultivar I-488 and the resistant cultivar T-173.

	Cultivar I-488 (S)						Cultivar T-173 (R)					
	5A	M1	M2	M3	M4	M5	5A[e]	M1	M2	M3	M4	M5
IPF	5.00	5.20	5.06	5.06	5.00	5.06	-	6.33	6.28	6.80	6.20	6.73
LP1	9.00	8.46	8.40	8.33	8.00	8.33	-	11.26	10.93	12.26	11.60	12.26
LP50	12.13	10.60	10.80	10.86	10.66	10.80	-	14.40	15.30	16.20	16.20	15.73
ULD[d]	8.68	5.66	6.07	5.73	7.12	6.48	0.00	3.34	5.43	4.68	5.14	5.74
UPD	5.04	2.17	2.48	2.23	3.42	2.84	0.00	0.57	1.50	1.12	1.33	1.69
USM[d]	7.08	6.06	6.31	5.95	6.87	6.68	0.00	4.91	5.91	5.94	5.81	5.88

a Each value is a mean of 15 observations; please see table 6.4 for significance of difference between isolates and Table 6.2 for descriptions of aggressiveness traits.
b Isolates sharing the same alphabet within a trait do not differ significantly ($P < 0.05$).
c The six aggressiveness traits were incubation period to flecking (IPF), latent period to eruption of first uredenium (LP1), latent period to production of 50% uredinia (LP50), cumulative uredinial number per leaf disk (ULD; square root transformed), uredinial number per day (UPD), and urediniospores produced per square mm (USM; Log + 1 transformed).
d Transformed data has been presented.
e Race 5A was avirulent on cultivar T-173.

Table 6.4 Ranking of the wild type race 5A and the five radiation induced virulent mutant isolates of M. medusae in the decreasing order for six aggressiveness traits, on susceptible (I-488) and resistant (T-173)[a] cultivar of poplar[b].

	High Aggressiveness ----------> Low Aggressiveness											
IPF	5A[a]	5M4[a]	5M2[a]	5M1[a]	5M3[a]	5M5[a]	**5M4[b]**	**5M2[b]**	**5M1[b]**	**5M5[b]**	**5M3[b]**	
LP1	5M4[a]	5M3[ab]	5M5[ab]	5M2[ab]	5M1[b]	5A[c]	**5M2[d]**	**5M1[d]**	**5M4[de]**	**5M5[e]**	**5M3[e]**	
LP50	5M1[a]	5M4[a]	5M2[a]	5M5[a]	5M3[a]	5A[b]	**5M1[c]**	**5M2[d]**	**5M5[de]**	**5M3[e]**	**5M4[e]**	
ULD[c]	5A[a]	5M4[b]	5M5[bc]	5M2[c]	5M3[cd]	5M5[cde]	5M1[d]	**5M2[de]**	**5M4[e]**	**5M3[f]**	**5M1[g]**	**5A[h]**
UPD	5A[a]	5M4[b]	5M5[c]	5M2[cd]	5M3[d]	5M1[d]	**5M5[e]**	**5M2[e]**	**5M4[e]**	**5M3[e]**	**5M1[f]**	**5A[g]**
USM[c]	5A[a]	5M4[ab]	5M5[bc]	5M2[cd]	5M1[d]	5M3[de]	**5M3[de]**	**5M5[e]**	**5M2[e]**	**5M4[e]**	**5M1[f]**	**5A[g]**

[a] Isolates appearing in normal print are for susceptible cultivar(I-488) and in **bold** print are for resistant cultivar (T-173).
[c] Isolates sharing the same alphabet within a trait do not differ significantly ($P < 0.05$); race 5A has been underlined for better contrast.
[c] Using transformed values.

Figure 6.1 Disease progress curves (basis, uredinia produced per leaf disk) for wild type race 5A and its five radiation induced virulent mutatants, on susceptible cv. I-488 and resistant cv. T-173.

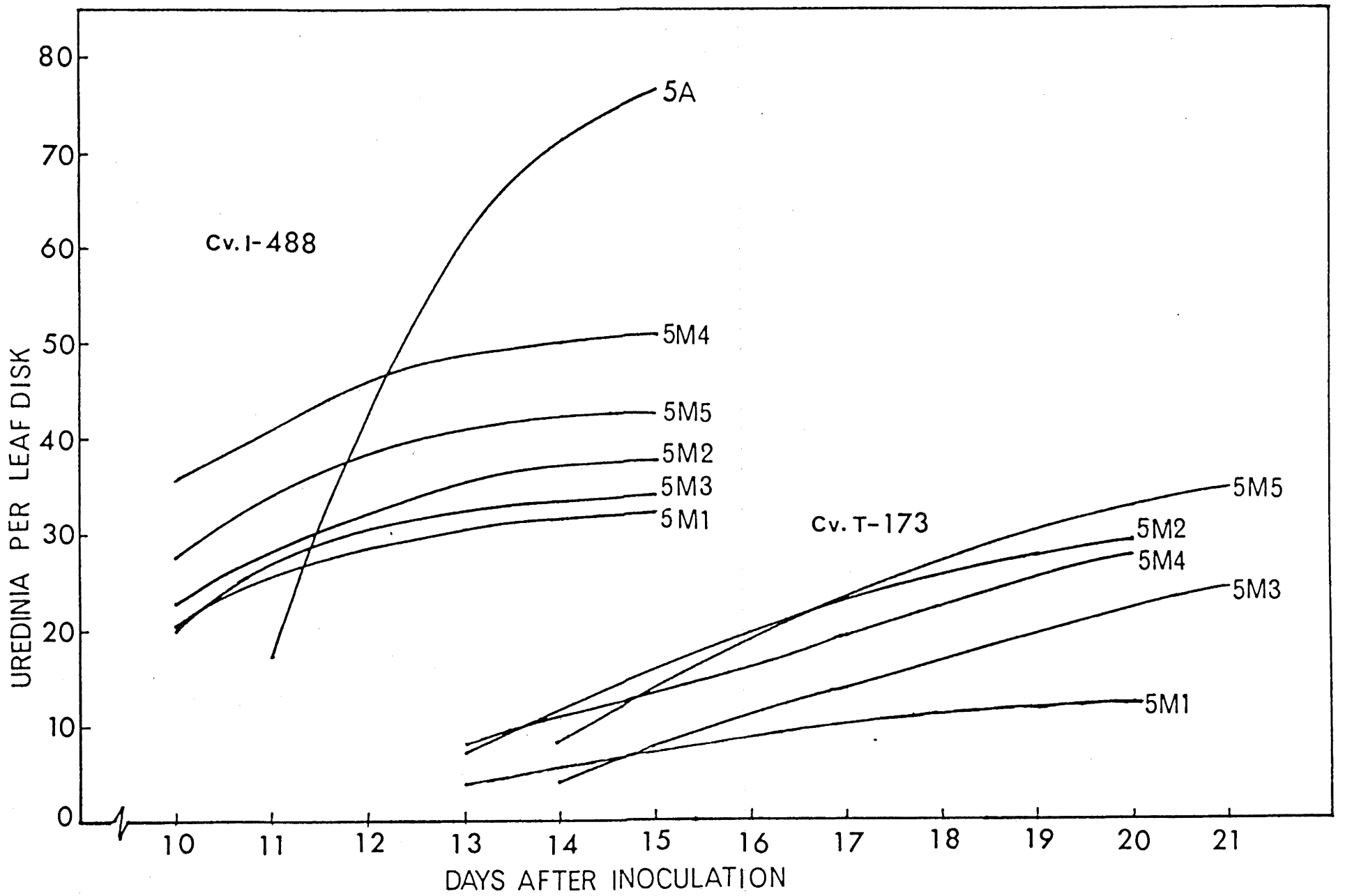

80
70
60
50
40
30
20
10
0
URED INIA PER LEAF DISK
Cv. I-488
5A
5M4
5M5
5M2
5M3
5M1
Cv. T-173
5M5
5M2
5M4
5M3
5M1
10
11
12
13
14
15
16
17
18
19
20
21
DAYS AFTER INOCULATION

Plate 6.1 The reaction of races 5A and radiation induced virulent mutant isolate 5M2 of M. medusae on P. x euramericana cv. I-488 and P. deltoides cv. T-173.

Plate 6.2 The aggressiveness (uredinial number) of M. medusae race 5A and its five radiation induced virulent mutant isolates (5M1-5M5) on P. x euramericana cv. I-488.

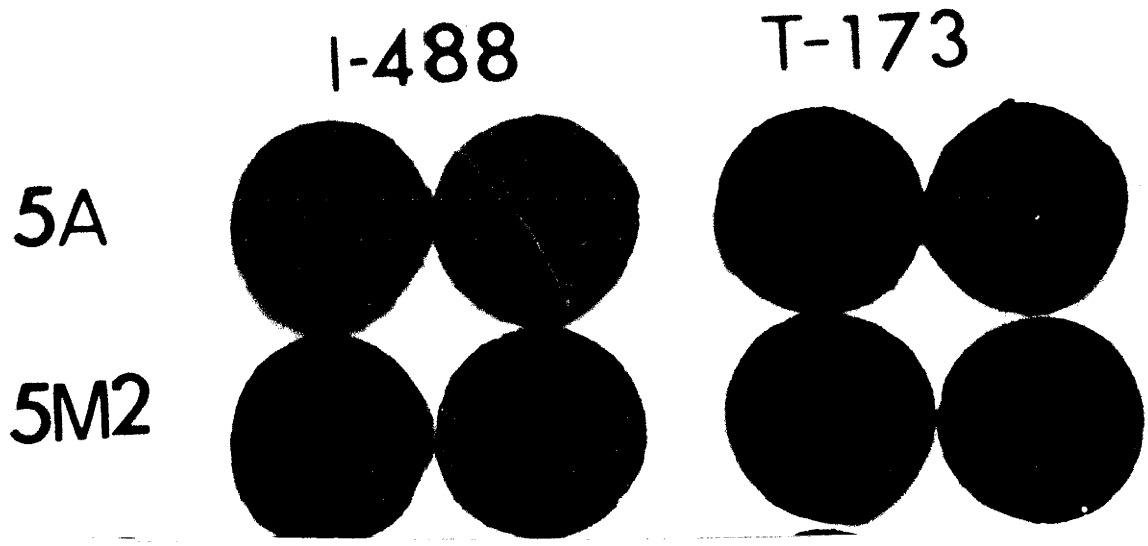


Plate 6.1

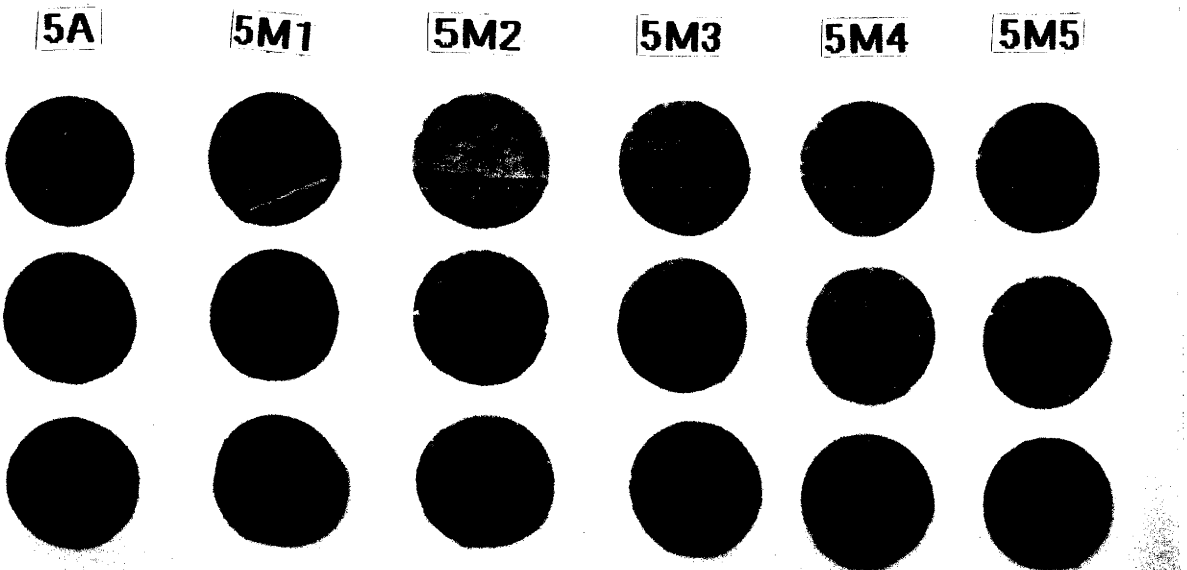


Plate 6.2

trait between the cultivars (Table 6.4). Such non-uniformity in ranking of mutants within a trait between the cultivars accounts for the significant cultivar x isolate interaction in ANOVA (Table 6.2), which in some instances involved reversal of ranking for aggressiveness. For example, on the basis of USM, 5M3 is the least aggressive isolate on cv. I-488 but the most aggressive mutant on cv. T-173. Similarly, 5M1 and 5M2 had the longest LP1 on cv. I-488 but shortest on cv. T-173 (Table 6.4).

On cv. I-488, on the basis of IPF, ULD and USM, race 5A was the most aggressive isolate, however in terms of LP1 and LP50 the mutants were more aggressive than 5A (Table 6.3 and 6.4). For all the traits of aggressiveness, the mutants, despite the significant differences between them, formed a cluster, distinctly removed from the value for race 5A (Table 6.4).

All three groups of curves for disease progress of the cultivar/isolate combinations (Fig. 6.1) differed significantly ($P < 0.01$) and thus the differences in ULD of the three groups are not artefacts of the time of final observation of ULD. The infectious period (from commencement of uredinial production till the end of the monocycle) for cv. I-488 was always shorter than that for cv. T-173. The ranking of the race/cultivar combinations for mean UPD (rate of disease progress) was 5A/I-488 (5.04) > mutants/I-488 (2.63) > mutants/T-173 (1.24).

Certain traits of aggressiveness for the mutants on both the cultivars were positively and significantly ($P < 0.05$) correlated. e.g. IPF and LP1 (0.93), LP50 and ULD (0.69), and between ULD and USM (0.56, $P < 0.1$) while IPF was negatively though not significantly, correlated with ULD, and USM (Table 6.5).

The irradiation dose under which each of the five mutants virulent on cv. T-173 was produced, was not consistently related to their ranking for aggressiveness for any trait.

6.3.2 STUDIES WITH NATURAL MUTANTS (EXPT. 6.2) : Races, cultivars and their interaction were significant ($P < 0.001$) sources of variation in the ANOVA for all the four traits of aggressiveness of the three races on the two cultivars (Table 6.6).

Table 6.5 Correlation coefficients between five aggressiveness traits (using means) among five radiation induced mutants isolate of M. medusae on cvs I-488 and T-173[a].

	IPF	LP1	LP50	ULD	USM
IPF	1.00				
LP1	0.93**	1.00			
LP50	0.11	0.39	1.00		
ULD	-0.28	0.01	0.69**	1.00	
USM	-0.21	-0.01	0.38	0.56*	1.00

[a] Degrees of freedom = 8.
** significant ($P < 0.05$), * significant ($P < 0.1$). All unmarked values are not significant ($P > 0.1$)

Table 6.6 Analysis of variance[ab] for race 4B and natural virulent mutants for four aggressiveness traits[c] on cvs I-488 and T-173.

Source	D F	IPF	LP1	ULD	USM
Isolate	2	3.41	84.90	32.08	3.59
Cultivar	1	44.10	376.75	288.45	53.66
Isolate X Cultvr	2	1.23	9.09	82.18	6.89
Residual	84	0.07	0.96	1.06	0.18
Total	89	0.66	7.55	6.81	1.22[†]

[a] Mean sum of squares.
[b] All the variances are highly significant ($P < 0.001$).
[c] The four aggressiveness traits are incubation period to flecking (IPF), latent period to production of first uredinium (LP1), number of uredinia per leaf disk (ULD; square root transformed), and number of urediniospores produced per square mm of leaf area (USM; Log_e transformed).
[†] Residual and total degrees of freedom for USM are 66 and 71 respectively.

Table 6.7 The means[a] for four aggressiveness traits of race 4B and natural virulent mutants of M. medusae on cvs I-488 and T-173.

	I-488 (S)			T-173 (R)		
	4B	4M1	4M2	4B	4M1	4M2
IPF	5.06	4.76	4.80	6.93	6.00	6.00
LP1	9.20	7.20	7.27	14.54	10.47	10.93
ULD	9.37	8.57	7.89	1.97	6.77	6.36
USM	6.90	6.81	6.45	3.95	5.53	5.51

The significance of differences between the isolates and the description of the aggressiveness traits are presented in Table 6.8.

Table 6.8 Ranking of the race 4B and the two natural virulent mutants of M. medusae in the decreasing order of aggressiveness on cvs I-488 and T-173 [abc]

High aggressiveness ----------> Low aggressiveness						
IPF	4M1[a]	4M2[a]	4B[b]	**4M1[c]**	**4M2[c]**	**4B[d]**
LP1	4M1[a]	4M2[a]	4B[b]	**4M1[c]**	**4M2[c]**	**4B[d]**
ULD	4B[a]	4M1[b]	4M2[b]	**4M1[c]**	**4M2[c]**	**4B[d]**
USM	4B[a]	4M1[a]	4M2[b]	**4M1[c]**	**4M2[c]**	**4B[d]**

[a] The values in the **bold** print are for cv. T-173, and those in the normal print are for cv. I-488.

[b] Isolates sharing the same alphabet within a trait do not differ significantly ($P > 0.05$)

[c] The traits measured were incubation period to flecking (IPF), latent period to production of first uredinium (LP1), number of uredinia per leaf disk (ULD; square root transformed), and number of urediniospores produced per square mm of leaf (USM; Log_e transformed).

The ranking of the races for aggressiveness on the susceptible cultivar I-488 depended on the parameter employed; thus while race 4B was most aggressive for the amount of disease produced (ULD and USM), it was the least aggressive race for the timing of disease expression (IPF and LP1) (Tables 6.7 and 6.8). In contrast, on the resistant cv. T-173, irrespective of the trait of aggressiveness, 4B was the least aggressive race. For example, race 4B had very long LP1- 14.5 days compared to *c.* 10 days for the races 4M1 and 4M2 and produced less than four uredinia per leaf disk (mean) compared to *c.* 40 uredinia produced by the latter races (Table 6.7). Due to the transformations of ULD and USM, the apparent differences between the races for these traits appear smaller in Table 6.7 than their absolute values indicate.

For all traits of aggressiveness on both cultivars, race 4B differs signficantly from races 4M1 and 4M2 but these latter two races differ signifcantly only for USM on cv. I-488 (Table 6.8). Thus for most traits on both cultivars, the virulent races 4M1 and 4M2 (races with broader virulence spectrum; Chapter 5) are distinct from that of the avirulent race 4B (with relatively narrow virulence spectrum; Chapter 5), the latter being more aggressive for disease severity on the susceptible cultivar.

The major results were reproduced when the experiment was repeated using one mutant line (5M2) and race 5A on both the cultivars.

6.4 DISCUSSION

Both the mutant series were selectively isolated for their compatibility on cv. T-173. As both the mutant groups have been derived from the avirulent races (either 5A and 4B), they can be expected to share common genetic background with their respective wild types. In contrast the cultivars are of disparate geographic origin (I-488 - northern Italy; T-173 - Texas, USA), hence their major contribution to variation in all traits of aggressiveness is not unexpected (Table 6.2 and 6.6).

6.4.1 RELATIONSHIP OF INCREASED VIRULENCE SPECTRA AND AGGRESSIVENESS OF ISOLATES ON THE SUSCEPTIBLE CULTIVAR

Aggressiveness of the mutants, vis a vis that of the wild types (races 5A and 4B), on the susceptible cultivar I-488, appears to increase (shorter LP1 and LP50) or decrease (reduced ULD and USM) depending on the traits considered (Table 6.3, 6.7).

The mutant isolates, both natural and radiation induced, are strikingly less aggressive on cv. I-488 (longer IPF, reduced ULD, UPD and USM) than wild types (Figs 6.2, 6.3). This reduced aggressiveness of disease severity traits in the mutants is associated with their acquisition of virulence, on cv. T-173 and certain other P. deltoides cultivars (Chapter 5), ie. factor/s unnecessary for compatibility on cv. I-488. This suggests a negative relationship between the increased virulence spectra of these isolates and their aggressiveness on a universal suscept. Similar decrease in aggressiveness (basis ULD) of mutant isolates were observed on another universal suscept, cv. 'italica' (Chapter 5). The minor, albeit significant, differences among the irradiated mutant isolates may be due to the cryptic changes in loci other than those concerned with virulence and is supported by the consistency with which, in a rank order test for any trait of aggressiveness, the mutants form a cluster distinct from the values of the wild types (Tables 6.2 amd 6.5). In these results, the overall virulence/aggressiveness relationship suggests that, while biotypes with differing aggressiveness could occur within a race, the major differences in aggressiveness appear to be associated with the virulence/avirulence make-up of the genotype; such an observation is also supported from the results of Chapter 5.

Certain aggressiveness factors may be linked to avirulence genes and hence the deletion of a chromosomal segment would result in reduced aggressiveness. Linkage of major genes with those determining quantitative traits are known in other organisms (Thompson and Thoday, 1979). Alternatively, avirulence genes themselves may behave additively as aggressiveness factors in the absence of corresponding resistance factors (Person and Mayo, 1974) in the host and hence, mutation of such

avirulence alleles which increases the spectrum of virulence, would result in reduced aggressiveness. The present results also suggest that high aggressiveness may be dominant to low aggressiveness, as mutations are usually from dominant to recessiveness, and these traits may not be under very complex genetic control. Emara & Sidhu (1974) also found evidence for dominance of aggressiveness in Ustilago hordei (pers.) Lagerh. crosses but two thirds of the genetic variance was found to be additive.

Reduced aggressiveness associated with increased virulence ('cost of virulence', Leonard, 1977; Pryor, 1977) could result in a competitive advantage to simpler races (sensu narrow virulence spectrum) on simple cultivars (having few resistance genes) and help the pathosystem to achieve equilibrium at intermediate gene frequencies (Leonard, 1977; Leonard and Czochor, 1980). Leonard (1979) compared the isolines of race 0 and T of Bipolaris maydis (Nisk.) Shoemaker and estimated that the cost of virulence in a virulent race T was 0.12 - 0.30. Flor (1971), from studies on M. lini concluded that dominant avirulence genes were associated with high aggressiveness. Teo and Baker (1975) also observed reduced infection type (IT 3) by the EMS induced virulent mutants of Puccinia graminis f. sp. avenae on many universal suscepts of oats, compared to the wild types (IT 4) from which they were derived. Nelson et al (1970) however, observed positive association between virulence spectra and aggressiveness in Exserohilum turcicum and suggested that both traits may be conditioned by the same genes, however their conclusion has been questioned by Johnson (1984).

The residual effect of the irradiation is not the cause of reduced aggressiveness observed in the mutants, because such effects result in a non-heritable, increased latent period in exposed urediniospores of M. medusae (Chapter 4) in contrast with reduced latent period in the mutants of the present study. Further, aggressiveness was not correlated with the dosage level at which the mutant was induced, and comparable reduction in ULD and USM was observed even in the natural mutants which had not been exposed to irradiation. Thus the reduced aggressiveness recorded here appears to result mainly from its association with increased virulence.

6.4.2 RELATIVE RESISTANCE OF CULITVARS I-488 AND T-173

The cultivar constitution was a significant contributor to variation in disease progression (Figs 6.1) as well as in the constituent traits of aggressiveness (Tables 6.2, 6.3, 6.6 and 6.7). Thus, although the mutants overcame the major gene resistance of cv. T-173 to the wild types, they were still confronted with an array of rate-reducing mechanisms in this cultivar. As a consequence of increased latent period and reduced rate of disease progression for the mutants on cv. T-173 (Tables 6.3 and 6.7, and Fig. 6.1), the initiation of the second cycle would be delayed by 4-5 days and the inoculum available be reduced by *c.* 30% compared with that of cv. I-488. This indicates an association between rate of disease progression and initial disease inoculum ($\frac{1}{2}x^{o}$) as proposed by Parlevliet (1979).

The rate reducing mechansims in cv. T-173 were not observed in, for example, *P.x euramericana* cv. I-154, the reaction of which changed from resistant (mesothetic) to race 5A, to highly susceptible to these mutants (Chapter 5). Thus the usefulness in a breeding program of a host cultivar containing a 'defeated' resistance gene probably depends on the background genotype (Skovamand *et al*, 1978; Eskes, 1983a; Parlevliet, 1983a) since the newly virulent gene may not necessarily enjoy preferential advantage (*sensu* fitness) on the corresponding resistance gene (cf. 'a' values, in Leonard and Czochor, 1980). Such an observation supports the suggestion of Leonard (1977) that a multiline cultivar should be more effective if it consists of a series of cultivars with different genetic backgrounds rather than a series of backcross lines from the same recurrent parent variety.

The quantitative traits studied here are both measures of disease expression in a monocycle and contributors to the progress of the host-pathogen interaction in a polycyclic epidemic. The present and other results with poplar leaf rust (Heather and Chandrashekar, 1982) indicate that such quantitative specificity may have a strong genetic base and support the integrated system of host pathogen relations proposed by Parlevliet and Zadoks (1977). The correlation coefficients between certain traits of aggressiveness confirm the observations of

Singh and Heather (1982a).

Watson (1970), and Leonard (1977), have suggested that for a virulent mutant to be epidemiologically successful, it should arise in an aggressive genotype. While the present results support this view, it suggests an association of increased virulence with reduced aggressiveness. Thus for a mutant to be epidemiologically successful on the susceptible culitvar, it should arise in a super-aggressive biotype in a moderately aggressive population so that, despite the cost of virulence or genetic load, it's fitness would be relatively higher than the mean fitness of the avirulent population. Parlevliet (1983a) considers the adaptation of the pathogen population to a new, previously resistant host as a two-stage process, initially of the acquisition of the matching virulence gene followed by a recovery in general fitness. Possibly the mutants tested here are still be in transient stage (sensu stage 1 of Parlevliet, 1983a) in recovering their general fitness. However, in the mutants, the interactive nature of the parasitic relationship observed, and the negative association of the increased virulence and aggressiveness components, suggest that continuing selection for higher aggressiveness on cv. T-173 may lead to further loss of aggressiveness on cv. I-488 and vice versa. Such mutually exclusive behaviour of fitness traits has been demonstrated in other systems (Triantaphyllou, 1975; Hiura, 1978; Wolfe, 1984; Alexander et al, 1984a; Leonard, 1984) and may be important factors in pathosystem stability. Occurrence of such non-random, disassociation or opposing trends for aggressiveness can be tested by serial culturing of the isolates on both cultivars.

The epidemiological significance of the changes recorded here within a monocycle, requires further experimentation in a continuous polycyclic disease situation, particularly as the changes in aggressiveness associated with increased virulence spectra is not unidirectional for all the traits (cf. LP1 and ULD). Thus, although the mutants are expected to be relatively less fit to survive on the susceptible cultivar in competition with the wild type because of their reduced infection and sporulation, it is possible that they may persist in low frequencies because of their advantage in shorter latent period. Also, the present results were confined to the study of aggressiveness, of both virulent and avirulent races in isolation and in

a single monocycle. Information on association or disassociation on aggressiveness and on the relative fitness of the mutant races in relation to competition with the wild types, can be obtained by race mixture and serial culture studies, and has been presented in Chapter 8.

6.5 SUMMARY

The radiation induced and natural virulent mutants of M. medusae were compared for their aggressiveness with their respective wild type races on a susceptible (I-488) and a resistant (T-173) cultivar of poplar. On cv. I-488, the wild type was more aggressive for uredinial and urediniospore production, while the mutants had shorter latent period. The cv. I-488 was more susceptible than cv. T-173 to all the mutant isolates for all traits, and cv. T-173 was characterised by slower disease progression, reduced infection and sporulation. The results suggest an association of increased virulence spectrum in the mutants with reduced aggressiveness on a universal suscept, while the fitness of the mutants on resistant cultivar possibly depends on the genetic background of the host.

CHAPTER 7

VARIABILITY AND DIRECTIONAL SELECTION FOR ENHANCED AGGRESSIVENESS TRAITS IN *MELAMPSORA MEDUSAE* BY HOST GENOTYPE

7.1 INTRODUCTION

An understanding of selection toward maximal expression of disease, within a race by host genotypes, is pertinent to prediction of potential disease impact and stability of resistance. This is especially true in the case of perennial crops, due to relative constant host genotype composition, often over a considerable area, and over an extensive crop rotation time. Hill and Nelson (1982) have suggested that, due to the high heritability of certain aggressiveness or parasitic fitness traits, the pathogen can respond to selection for increased aggressiveness on compatible cultivars. Aggressiveness in some plant pathogens has been recognised to be polygenically inherited (Emara and Sidhu, 1974; Leonard, 1975; Brasier, 1977).

In Australia, *M. medusae* appears to reproduce only asexually and thus, any change in its aggressiveness would result mainly from mutation and possibly, other asexual mechanisms. This chapter reports a study on variability and directional selection for enhanced aggressiveness (five traits) in two races of *M. medusae*, on two cultivars of poplars. The experiment involved both natural and irradiated (^{60}Co) populations within a race and serial culturing for more than eleven generations on detached leaves of *P.* x *euramericana* cv. I-488.

7.2 MATERIALS AND METHODS

7.2.1 THE CULTIVARS, THE PATHOGEN RACES AND THE IRRADIATION: Clonal plants of *P.* x *euramericana* cvs. I-488 and I-214, were grown in a rust-

free glass house and leaves of uniform age (c. three months old) were used throughout the study (section 2.2.2.1). Though differences in quantitative response of these two cultivars to races of M. medusae have been observed, both cultivars are universal suscepts as they do not show 'hypersensitive' type resistance to any of the recognised races of this pathogen (Singh and Heather, 1982b; Prakash and Heather, unpublished).

Spores of races 4B and 8A (section 2.3.1) were multiplied initially on surface sterilised, detached leaves of cv. I-488 (at least five cycles), to produce fresh urediniospores (section 2.4.1). A portion of these spores from each race was exposed to 400 Gy of gamma irradiation from a ^{60}Co source (a near LD50 dose cf. Chapter 4), and subsequently, both irradiated and non-irradiated spores were dried *in vacuo* over silica gel (12 h) and P_2O_5 (12 h).

Urediniospores of both populations of each race were inoculated (10 mg - irradiated, 5 mg - non-irradiated) independently on surface sterilised, detached leaves of cv. I-488, and incubated in plastic Petri dishes at 'standard environment' (section 2.5.1). The increased spore load for irradiated population was necessary to compensate for the c. 50% lethality expected in spores exposed to 400 Gy irradiation (Chapter 4).

7.2.2 THE SERIAL CULTURING OF RACES ON CV. I-488 : The populations of both races were serially cultured on cv. I-488 for eleven generations, after harvesting on 15 or 16 th day (post-inoculation) at each generation. A sample of spores from each generation was separately dried and stored (-14°C). At the end of 11 generations, spores representing 1, 6 and 11th generations were separately multiplied on cv. I-488, to produce fresh, dry spores of each of the three generations for experimental inoculations. Although the spores actually constituted 2, 7 and 12th generations, they are referred to as representing 1, 6 and 11 generations of serial culture. Thus, the first generation populations of both races had undergone two generations of initial culture in the study.

7.2.3 EXPERIMENTAL EVALUATION OF AGGRESSIVENESS: Urediniospores (4 mg) of isolates [representing 2 populations (irradiated and non-irradiated) X 2 races X 3 generations] were deposited separately on leaf disks (15/replicates per treatment) of cvs. I-488 and I-214, and on a few coverglasses. Uniformity of deposition and germination potential of urediniospores was checked on the coverglasses. Inoculated leaf disks were placed on plastic foam, soaked with GA solution (10 mg/L), sealed in glass Petri dishes and incubated in control growth cabinets at 'standard environment'.

7.2.4 OBSERVATIONS AND ANALYSIS: Five aggressiveness traits were recorded on the replicate leaf disks, viz. incubation period to flecking (IPF), latent period to production of first uredinium (LP1), uredinia produced per leaf disk (ULD), urediniospores produced per square mm of leaf area (USM), and urediniospores produced per uredinium (USU) (section 2.6.2). Shorter IPF and LP1 and higher ULD, USM and USU are indicative of high aggressiveness of an isolate on a cultivar.

The data were tested for homoscedasticity and normality of error variance (Neter and Wasserman, 1974) and values for ULD, USM and USU were transformed appropriately to fulfill the above requirements. The analysis of variance was performed on the factorial design (2 X 2 X 2 X 3). The significance of differences between the means of main effects were computed using the standard error of differences (SED) from appropriate main effects (Table 7.2), while the cell means of all the treatments were tested with SED of the third order interaction component.

7.3 RESULTS

7.3.1 THE ANALYSIS OF VARIANCE : A summary of the variance ratios for each trait is presented in Table 7.1. Irrespective of the parameter employed to assess disease development, cultivar, race and number of generations were highly significant in partitioning the variation within the experimental data ($P < 0.001$). Although the population (irradiated or non-irradiated) was a significant source of variation in traits of disease severity (ULD, USM and USU, $P < 0.001$), it was not significant

Table 7.1 Analysis of variance[a] for five traits of aggressiveness[b] by two races of *M. medusae* cultured on cv. I-488 for 11 generations; results of factorial study of two races on two cultivars of *P.* x *euramericana*, representing 1, 6 and 11th generations, and two populations (irradiated and non irradiated).

Variation Source	D F	IPF	LP1	ULD	USM	USU
Cultivar (C)	1	183.47	248.33	629.59	146.04	14.17
Race (R)	1	123.67	42.02	42.64	0.55	2.62
Generation (G)	2	2.58	7.01	40.78	223.19	49.40
Population (P)	1	0.22^{ns}	0.02^{ns}	22.86	0.45	0.89
C X R	1	4.22	4.67	0.96^{ns}	2.81	2.46
C X G	2	0.91	0.41^{ns}	2.25	1.92	1.35
R X G	2	1.27	0.10^{ns}	8.28	0.19^{ns}	0.26*
C X P	1	0.47^{ns}	2.02	5.98	0.71	0.02^{ns}
R X P	1	1.01	0.22^{ns}	1.42	2.44	1.97
G X P	2	0.17^{ns}	0.10^{ns}	1.95**	0.14^{ns}	0.28^{ns}
C X R X G	2	1.15	0.14^{ns}	2.97	0.31	0.73
C X R X P	1	0.07^{ns}	2.33	3.95	2.62	5.24
C X G X P	2	0.48*	0.03^{ns}	1.42*	0.07^{ns}	0.12^{ns}
R X G X Pn	2	0.17^{ns}	2.53	21.83	0.56	0.53
C X R X P	1	0.07^{ns}	2.33	3.95	2.62	5.24
C X G X P	2	0.48*	0.03^{ns}	1.42*	0.07^{ns}	0.12^{ns}
R X G X P	2	0.17^{ns}	2.53	21.83	0.56	0.53
C X R X G X P	2	0.03^{ns}	0.71^{ns}	0.44^{ns}	0.18^{ns}	0.25*
Residual[c]	336	0.15	0.27	0.41	0.06	0.07
Total[c]	359	1.05	1.15	2.86	0.73	0.21

[a] All unmarked values are highly significant ($P < 0.001$). ** - $P < 0.01$, * - $P < 0.05$, ns - not significant.
[b] The details of traits of aggressiveness are presented in Table 7.2
[c] For USU and USM, the residual degrees of freedom is 264 and total degrees of freedom is 287.

in the timing of disease expression (IPF and LP1, $P > 0.05$). Though cultivars contributed most and population the least, to the variation in all traits, race was a relatively more significant source of variation compared to generation for IPF, LP1 and ULD, while generation were relatively more important for USM and USU. When the variances due to interactions were added to the residual and the main effects recalculated, the level of significance of main effects did not change and thus the main effects have been considered independently (section 7.3.2.) of the interactive effects.

The first and second order interaction were usually significant sources of variation, particularly for traits of disease severity (ULD, USM and USU), but the third order interaction was significant for only USU (Table 7.1). The significance ($P < 0.001$) of cultivar x race component (generation and population effect held constant) for all traits except ULD, suggests a degree of quantitative specificity of the two races on the two cultivars (Table 7.1). The significant cultivar X generation is indicative of differences in susceptibility of cultivars over generations while the race X generation interaction suggests that there were also differences in the level of aggressiveness of races over generations. Similarly race X population and generation X population interactions indicate the differential effect of irradiation between races and between generations (Table 7.1).

The significance of second order interactions suggest, that for traits of disease severity, at least some of the specific two-factor interaction of any two variables differ, depending on the level of the third variable. Thus, in addition to the comparison of the main effects, it is necessary to identify, within each race-population, the trend of changes in aggressiveness across generations separately for both cultivars (section 7.3.3).

7.3.2 TRENDS ACROSS THE MAIN EFFECTS: CULTIVARS, RACES, GENERATIONS AND ISOLATES : The mean values for four main effects (with fixed interaction effect) are presented in Table 7.2. As stated previously, due to the considerable significance of interaction effects (Table 7.1;

Table 7.2 Means for five traits of aggressiveness [a] for cultivars, races, generations and isolates (main effects).

Trait	Cultivar		Race		Generation			Isolates	
	I-488	I-214	4B	8A	1	6	11	Non-Irrd	Irradiated
IPF (days)	5.47	6.89	5.59	6.76	6.31[a]	6.20[a]	6.02	6.15	6.20
LP1 (days)	8.18	9.84	8.67	9.35	9.25	9.01	8.77	9.02[a]	9.06[a]
ULD[b]	6.89	4.25	5.91	5.23	5.03	5.49	6.19	5.32	5.82
USM[b]	7.08	5.66	6.41	6.32	5.98	6.37	6.77	6.33	6.41
USU[b]	8.36	7.91	8.04	8.23	7.93	8.15	8.33	8.19	8.08

[a]The five aggressiveness traits are incubation period to flecking (IPF), latent period to production of first uredinium (LP1), uredinia produced per leaf disk (ULD), urediniospores produced per square mm of leaf (USM) and per uredinium (USU).

[b] Square root transformed values were used for ULD, while $\log_e + 1$ transformed values were used for USM and USU.

section 7.3.1), main effects can be considered for indication of their trends but more precise interpretation of the data requires inclusion of the interactive effects (section 7.3.3.; Table 7.3).

For all traits, cv. I-488 was relatively more susceptible than cv. I-214. With the exception of USU, race 4B was more aggressive than race 8A for all traits. Across generations, a systematic increase in aggressiveness for all traits could be observed from 1 to 6, and 6 to 11 generations. Although significant (except for LP1), irradiation was less important than other main effects as a cause of variation in the traits. While the non-irradiated population was more aggressive for IPF and USU, the irradiated population was more aggressive for ULD and USM.

The general consistency in ranking of all main variables (e.g. cultivars or races) across most traits (except USU) suggests that aggressiveness levels observed within a monocycle, are indicative of the epidemiological fitness of the isolates and not due to artefacts of the traits chosen.

7.3.3 TRENDS ACROSS GENERATIONS BY POPULATIONS WITHIN TWO RACES ON BOTH CULTIVARS : The level of aggressiveness (five traits) for 1, 6 and 11 generations of non-irradiated and irradiated popualations of races 4B and 8A on cvs I-488 (on which they had been cultured) and I-214, are presented in Table 7.3.

Note: In the following presentation, the specific population (non-irradiated or irradiated) is mentioned only when there are marked differences in the trends between them. Also, the level of significance employed is P = 0.05, and this value is not repeated when discussing differences between treatments.

7.3.3.1 INCUBATION PERIOD TO FLECKING (IPF) : With race 4B, irrespective of the cultivar, generation or irradiation, IPF was not significantly affected. However, the aggressiveness of race 8A increased (decreased IPF) across generations on cv. I-214, but not on cv. I-488.

Table 7.3 Means[a] of five aggressiveness traits across 1, 6 and 11 generations of non-irradiated and irradiated populations of races 4B and 8A of M. medusae on cvs I-488 and I-214 of poplar. Cell mean values sharing the same superscript within a population do not differ significantly ($P > 0.05$). For comparision of values across populations, races or cultivars, use the L.S.D. values.

a) **INCUBATION PERIOD TO FLECKING (IPF):**

CULTIVAR	RACE & POPULATION	GENERATIONS 1	6	11
I-488				
	4B-NonIrrd	5.07^{a}	5.13^{a}	5.00^{a}
	4B-Irrd	5.00^{a}	5.00^{a}	4.73^{a}
	8A-Nonirrd	6.00^{a}	5.80^{a}	5.80^{a}
	8A-Irrd	6.00^{a}	6.00^{a}	6.00^{a}
I-214				
	4B-NonIrrd	6.13^{a}	6.40^{ab}	6.00^{a}
	4B-Irrd	6.26^{a}	6.26^{a}	6.13^{a}
	8A-NonIrrd	7.93^{c}	7.53^{b}	7.00^{a}
	8A-Irrd	8.13^{b}	7.47^{a}	7.47^{a}

$LSD_{0.05}$ - 0.283

b) **LATENT PERIOD TO PRODUCTION OF FIRST UREDINIUM (LP1) :**

CULTIVAR	RACE & POPULATION	GENERATIONS 1	6	11
I-488				
	4B-NonIrrd	8.13^{b}	8.0^{ab}	7.66^{a}
	4B-Irrd	8.26^{b}	8.00^{ab}	7.66^{a}
	8A-NonIrrd	9.06^{c}	8.53^{b}	8.20^{a}
	8A-Irrd	8.46^{b}	8.20^{ab}	8.00^{a}
I-214				
	4B-NonIrrd	9.33^{a}	9.53^{a}	9.26^{a}
	4B-Irrd	9.86^{b}	9.26^{a}	9.06^{a}
	8A-NonIrrd	10.60^{b}	10.06^{a}	9.86^{a}
	8A-Irrd	10.33^{a}	10.46^{a}	10.46^{a}

$LSD_{0.05}$ - 0.378

c) UREDINIA PRODUCED PER LEAF DISK (ULD) : (Using sq. root transformed values)

CULTIVAR	RACE & POPULATION	GENERATIONS 1	6	11
I-488				
	4B-NonIrrd	6.71^{b}	5.73^{c}	7.79^{a}
	4B-Irrd	6.73^{c}	7.66^{b}	9.13^{a}
	8A-NonIrrd	5.31^{c}	6.53^{b}	7.02^{a}
	8A-Irrd	6.91^{a}	6.71^{a}	6.51^{a}
I-214				
	4B-NonIrrd	4.37^{b}	4.46^{b}	5.57^{a}
	4B-Irrd	3.31^{c}	4.99^{b}	5.57^{a}
	8A-NonIrrd	3.07^{c}	3.95^{b}	4.37^{a}
	8A-Irrd	3.87^{a}	4.19^{a}	4.31^{a}

$LSD_{0.05}$ - 0.462

d) UREDINIOSPORES PRODUCED PER SQ. MM. OF LEAF (USM) : (Using log + 1 transformed values).

CULTIVAR	RACE & POPULATION	GENERATIONS 1	6	11
I-488				
	4B-NonIrrd	6.71^{b}	6.99^{a}	7.12^{a}
	4B-Irrd	6.79^{b}	7.18^{a}	7.37^{a}
	8A-NonIrrd	6.65^{c}	7.05^{b}	7.43^{a}
	8A-Irrd	7.05^{b}	7.29^{a}	7.34^{a}
I-214				
	4B-NonIrrd	5.17^{c}	5.39^{b}	6.30^{a}
	4B-Irrd	5.26^{c}	6.03^{b}	6.64^{a}
	8A-NonIrrd	5.22^{c}	5.72^{b}	6.19^{a}
	8A-Irrd	4.96^{c}	5.27^{b}	5.72^{a}

$LSD_{0.05}$ - 0.207

e) UREDINIOSPORES PRODUCED PER UREDINIUM (USU) : (Using log + 1 transformed values)

CULTIVAR	RACE & POPULATION	GENERATIONS 1	6	11
I-488				
	4B-NonIrrd	8.03^{b}	8.63^{a}	8.13^{b}
	4B-Irrd	7.90^{b}	8.23^{a}	8.08^{ab}
	8A-NonIrrd	8.44^{b}	8.42^{b}	8.75^{a}
	8A-Irrd	8.31^{b}	8.62^{a}	8.83^{a}
I-214				
	4B-NonIrrd	7.35^{c}	7.66^{b}	8.26^{a}
	4B-Irrd	7.96^{b}	7.92^{b}	8.31^{a}
	8A-NonIrrd	8.07^{b}	8.08^{b}	8.45^{a}
	8A-Irrd	7.37^{c}	7.60^{b}	7.92^{a}

$LSD_{0.05}$ - 0.224

7.3.3.2 LATENT PERIOD TO PRODUCTION OF FIRST UREDINIUM (LP1) : On cv. I-488, both races 4B and 8A had significantly increased their aggressiveness for latent period by 11th generation. On cv. I-214, such an increase in aggressiveness was apparent for only race 4B (irradiated) and race 8A (non-irradiated), and was significant between the 1 generation and 6 or 11 generations.

7.3.3.3 UREDINIA PRODUCED PER LEAF DISK (ULD) : Both populations of race 4B and non-irradiated population of race 8A had significantly higher aggressiveness for ULD on both cultivars at 11, compared to the 1 generation. However, race 8A (irradiated) decreased its aggressiveness on cv. I-488 but increased on cv. I-214 over generations, although such changes were not significant. The reason for lower level of significance of 6th generation isolate of race 4B (irradiated) on cv. I-488 is not clear.

7.3.3.4 UREDINIOSPORES PRODUCED PER SQUARE MM OF LEAF (USM) : Both races increased their USM with increase in number of generations on both the cultivars, but such changes were more pronounced on cv. I-214 than on cv. I-488; On the latter, the changes in aggressiveness of the races were usually greater between 1 and 6 generations than between 6 and 11 generations.

7.3.3.5 UREDINIOSPORES PRODUCED PER UREDINIUM (USU) : The pattern of changes across generations in race 4B on cv. I-488 was relatively inconsistent for this trait, with increase in aggressiveness from 1 to 6 generations and decrease in aggressiveness from 6 to 11 generations. Such decrease in USU at 11 generation may be due to the larger number of uredinia observed for this race. But race 8A exhibited increase in aggressiveness with increase in number of generations. On cv. I-214, both races had significant increase in aggressiveness with increase in number of generations.

The aggressiveness of the irradiated population (all traits) was considerably less when directly inoculated at the beginning of the study (cf. Chapter 4) but as the isolates representing the first generation had actually been multiplied twice, they did not exhibit any

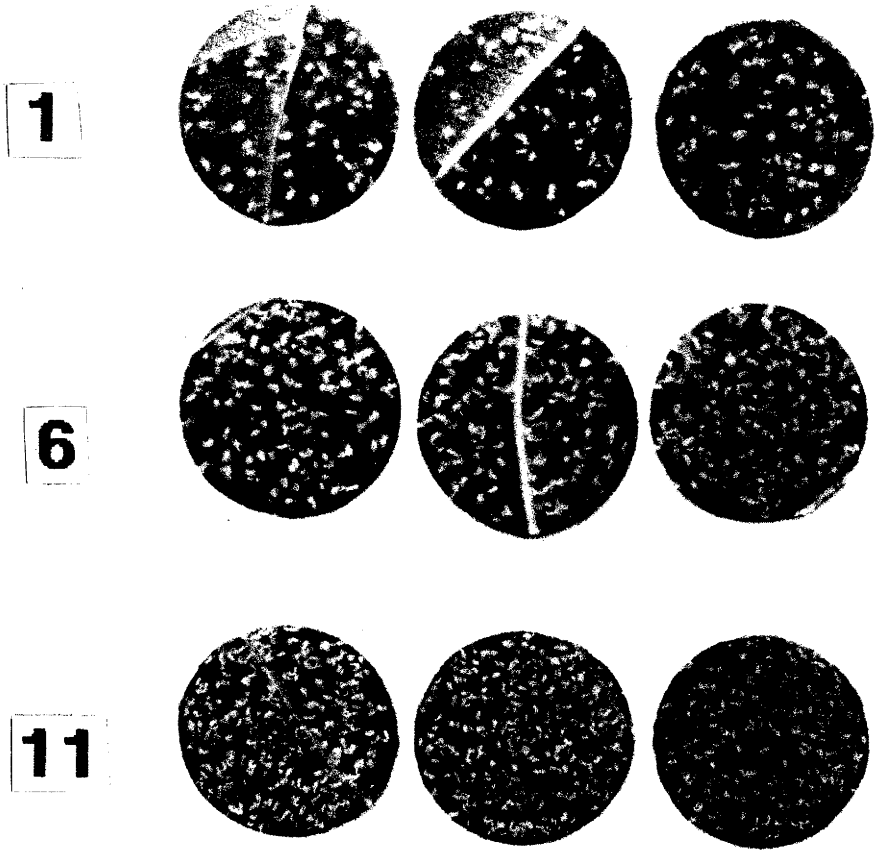


Plate 7.1 The aggressiveness (uredinial number) of isolates representing 1, 6 and 11 generations (serial culture) of race 4B (prior-irradiated), on P. x euramericana cv. I-488.

residual effects of irradiation (Table 14.2)

7.4 DISCUSSION

According to Hill and Nelson (1983), expectation of genetic advance under selection depends upon selection pressure, amount of genetic variation within the population, the magnitude of differences between the genotypic values and the heritability of variation. In the present study, as selection was not directly excercised on any of the traits, it is reasonable to assume that much of the selection pressure was by the host. In the irradiated population, quantitative mutations, which extend the genotypic flexibility for certain aggressiveness traits (ULD and USM) may have occured. This would have increased the amount of genetic variation in the population, and thus, could have been selected upon. According to Mukai (1979), the effect of polygenic mutation in the adaptation of the organisms has been less understood.

The present study may possibly explain the basis of observations in other plant pathogens, where isolates from a specfic cultivar were more aggressive on the cultivars from which they were isolates compared to other cultivars (Caten, 1974; Clifford and Clothier, 1974; Wolfe and Barrett, 1980). Several workers have shown experimentally that repeated culturing of pathogen isolates on specfic compatible hosts could increase their aggressiveness on those hosts (Cherwick, 1958; Caten, 1974; Triantaphyllou, 1975; Wolfe and Knott, 1982) and such a response may be due to the high heritability of the particular aggressiveness traits (Hill and Nelson, 1983,; Oard and Simons, 1983). Also, such rapid response to selection, suggests that these aggressiveness traits may be predominantly under additive genetic control, as observed in Ustilago hordei by Emara and Sidhu (1974).

The present study demonstrates that a compatible cultivar of poplar can exert directional selection pressure for enhanced aggressiveness of certain traits in these two races of M. medusae. This is in conflict with Vanderplank (1978) who suggested that aggressiveness, being a 'non-race specific' parameter, does not increase

on host cultivars, and any such increase observed would have negligible epidemiological effects. An extrapolation from the present results suggest that, under favourable and generally uniform environmental conditions, the resistance expressed in a partially or quantitatively resistant host genotypes may decrease. Also, much of the response of cv. I-214 was generally similar to that of cv. I-488, the cultivar on which the isolates had been cultured. Such correlated responses of the cultivars in supporting the directional selection of aggressisveness in races, suggest that both cultivars may share some similar genotypic composition for resistance. This is not surprising as both cultivars are natural hybrids (P. x euramericana) of unknown clonal parentage selected in the Po valley of northern Italy (Thielges, 1984). Alexander et al (1984a) observed increase in the aggressiveness of the field population of the Uromyces appendiculatus (Pers.) Unger. on the cv. US#39 of bean, after serially culturing for five generations on the partially resistant cv. slim green. The proportionately higher rate of increase in aggressiveness observed on cv. I-214 may be due to the initial low aggressiveness of the isolates on this cultivar.

Rayner (1983) did not observe directional selection for increased aggressiveness of two races of M. larici-populina on P. deltoides Marsh. cv. 7-2. Possibly this resulted from the mesothetic nature of the reaction in this cultivar to the races and thus, cultivars may differ in exercising selection pressure for increased aggressivenes on races. Paxman (1963) could not observe any increase in aggressiveness of Phytopthora infestans (Mont.) de Bary on potato after 90 passages, while James and Fry (1983) after treating the base population of P. infestans with a chemical mutagen (NTG), could not observe any increase in their aggressiveness upon subsequent serial culture.

Although, an increase in aggressiveness of traits were observed upon serial culturing in both irradiated and non-irradiated populations, such an increase was generally more pronouned in the former (basis ULD and USM) (Table 14.2). This suggests a genetic basis for the observed aggressiveness traits. Further, the generally higher USM but lower USU values observed for irradiated compared to non-irradiated populations in certain instances (e.g. 11 generation isolates of race 4B

on cv. I-488; Table 14.3 d and e), indicate that an increase in spore production in irradiated population was achieved mainly by increase in the infection efficiency (i.e. ULD) and less by increased fecundity *per se* of the irradiated populations. Possibly, irradiation resulted in higher genotypic variance for infection efficiency than fecuncity, and or the selection pressure for the former trait was more effective than for the latter. Hill and Nelson (1982) observed higher heritability values for infection efficiency in *Helminthosporium maydis* Nisikado and Miyake race T on corn. Watson (1970) observed increased aggressiveness by treatment with EMS, a chemical mutagen, on *Puccinia graminis* f. sp. *tritici*. Habgood (1976) suggested that the observed increase in aggressiveness of *Rhyncosporium secalis* (Oud.) Davis on barley hosts with no major genes for resistance, was a consequence of mutation.

7.5 SUMMARY

Two races (4B and 8A) of *M. medusae* were cultured (asexual) separately on cv. I-488 and isolates representing 1, 6 and 11 generations were tested for their traits of aggressiveness on cvs I-488 and I-214. Significant increase in the aggressiveness of the races were observed with an increase in the number of generations of serial culture. On cv. I-488, the magnitude of increase in the aggressiveness (ULD and USM) was slightly higher, when the base population of race 4B was irradiated (400 Gy) prior to the serial culture. This suggests the occurrence of mutation for these quantitative traits. This pathogen can respond to selection pressure for increased aggressiveness on these cultivars suggesting that quantitative forms of resistance may erode over time.

CHAPTER 8

DISASSOCIATION OF AGGRESSIVENESS TRENDS AND SURVIVAL IN MIXTURES OF VIRULENT RACES OF *MELAMPSORA MEDUSAE*

8.1 INTRODUCTION

In previous studies, the association of virulence spectrum and aggressiveness, of certain races of *M. medusae*, was negative in some (Chapter 6), but positive in other, cultivars (Chapter 5). Also, races 4B and 8A were observed to increase in aggressiveness when serially cultured for few generations on cv. I-488 (Chapter 7). In some pathogens, such positive selection for aggressiveness on a cultivar has been observed to influence the aggressiveness on other, unrelated cultivars (Traintaphyllou, 1975; Wolfe and Barrett, 1980; Wolfe and Knott, 1982; Alexander *et al*, 1984a; Leonard, 1984). Such studies, which demonstrate positive and negative association of aggressiveness trends in the pathogen races on various cultivars, have possible implications in disease control by employing mixtures of those cultivars which support disassociation of aggressiveness trends in the pathogen.

Here, the virulent and avirulent races refer to those with broad and narrow virulence spectra, respectively (Chapter 4). Studies on mixtures, involving a virulent and an avirulent race, cultured for several generations on cultivar susceptible to both races permits assessment of dominance of either race. Information from such studies are relevant to pathogen evolution and population shifts due to host cultivar patterns, the aspects of which have been discussed elsewhere (Appendix 4).

Many competition studies with race mixtures employed a qualitative (virulence/avirulence), rather than quantitative (aggressiveness), analysis of the races. ie., the investigation tested only the dominance of either race without emphasising the aggressiveness traits of the races invovled. However, the latter traits may be

important as they determine the fitness and competitiveness of the component races during an epidemic. In some reports, a few aggressiveness traits of the component races were assessed to investigate the possible traits conferring competitiveness (Ogle and Brown, 1971; Falhati-Rastegar et al, 1983). However, during serial culturing, changes in aggressiveness traits may occur even within pure races (Chapter 7). Thus better estimates of the competitive fitness of the races can be made by recording traits of aggressiveness of both the races and racemixes over the course of serial culture, in addition to that of number of pustules generated on the cultivars by the races or racemixes.

This investigation aims (1) to assess possible disassociation of aggressiveness trends by a virulent race (race 5M2) when cultured for eleven generations on cultivars cvs I-488 and T-173; and, (2) to assess the survival of a virulent race (race 5M2) in competition with an avirulent race (race 5A) by serial culturing a mixture of the races (1:1 proportion) over eleven generations on a susceptible cultivar (I-488). The survival of the virulent race was determined by its aggressiveness on a resistant cultivar (T-173).

8.2 MATERIALS AND METHODS

The virulent race was serially cultured on both the susceptible and the resistant cultivar, whereas the wild type and the racemixture were cultured only on the susceptible cultivar. Finally, the isolates representing 1, 6 and 11 generations of each race and racemix were tested for their aggressiveness traits on both the susceptible (I-488) and the resistant (T-173) cultivars. In addition, changes in the infection type of the races and their mixtures on twelve different cultivars of poplar, were recorded over the course of their serial culture, and the results of this study are provided in Appendix 7. A similar study was conducted employing natural virulent mutant, race 4M and its wild type race 4B, and the results of this study are also presented in Appendix 7.

8.2.1 THE PATHOGEN RACES : Studies were conducted with the radiation induced mutant, 5M2 (referred to as 5M) and its wild type race 5A.

8.2.2 THE HOST CULITVARS: The virulent race (5M), avirulent race (5A) and their mixture were cultured on cv. I-488 for eleven generations, while the virulent race (5M) was also cultured separately on cv. T-173. The virulence spectrum of the generation isolates of races and their mixture were assessed on twelve cultivars (Appendix 7).

8.2.3 SERIAL CULTURE OF THE RACES AND THEIR MIXES: The inoculation sequence followed to serially culture the races and their mixture and subsequent testing of their aggressiveness, on two cultivars of poplars, is shown in Fig. 8.1. Fresh, dry urediniospores of the virulent mutant and the avirulent wild type races were obtained by initial multiplication on cv. I-488 (c. 6 generations) (section 2.4.1) . A uniform mixture (proportion 1:1) of races 5A and 5M was obtained by gently shaking a combination of 5 mg of urediniospores of each race on sterile aluminium foil. This mixture was subsequently inoculated onto the surface treated, detached leaves of cv. I-488 which were then incubated in plastic Petri dishes under 'standard environment' (section 2.5.1). At the end of the monocycle, when the uredinia were sufficiently mature and appeared fluffy, the urediniospores were collected, dried and mixed thoroughly. A portion of these spores were used subsequently to reinoculate the fresh leaves of cv. I-488, while a portion of the urediniospores was preserved in a freezer at -14°C. This process was repeated for further 10 generations on fresh leaves of this cultivar. Similar serial culturing was made for the individual races (10 mg urediniospores) in each inoculation on the appropriate cultivars (i.e. race 5A on cv. I-488 and race 5M on cvs I-488 and T-173) (Fig. 8.1).

At the end of eleven generations, urediniospores representing the 1, 6 and 11 generations of all the four serial cultures in each study (1 and 6 generations from the freezer) were multiplied again on the respective cultivars to produce fresh, dry urediniospores for assessment of the final aggressiveness and the competitive ability of the races. As race 5M had been cultured on both cultivars, it is

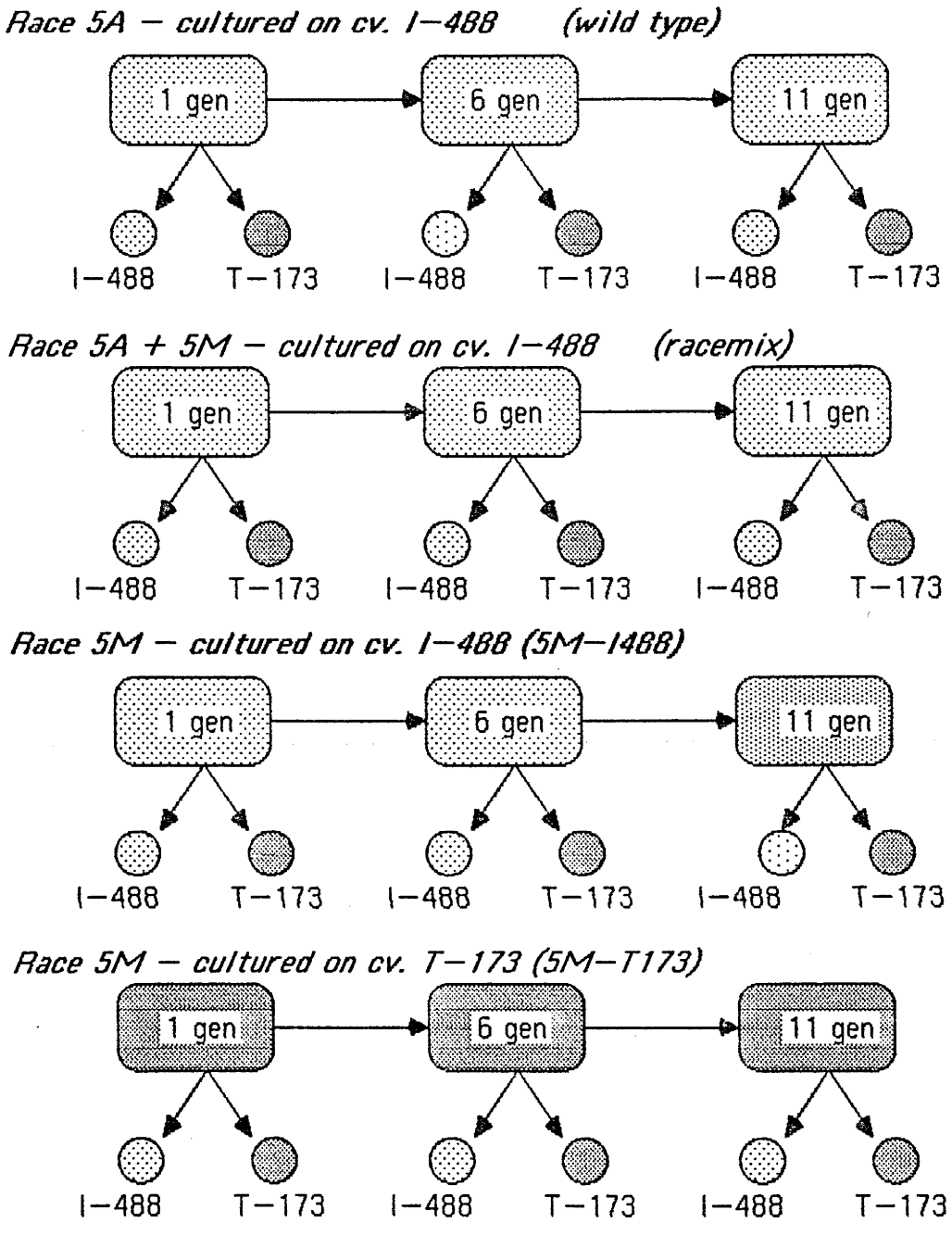


Figure 8.1 Schematic diagram of the serial culture of *M. meduse* races 5A, 5M and mixture 5A + 5M on cv. I–488, and of race 5M on cv. T–173; the races and the race mixture were serially cultured for 11 generations, and urediniospores representing 1, 6 and 11 generations were again multiplied, for one generation on appropriate cultivars and inoculated on leaf disks cvs. I–488 and T–173, to assess aggressiveness.

identified along with the cultivars on which it was cultured (i.e. race 5M-I488 or 5M-T173). As sufficient urediniospores of this race representing the first generation on cv. T-173 could not be obtained, those from cv. I-488 (1 gen) were treated as 5M-T173 (1 gen) [Thus, 5M-T173 (1 gen) and 5M-I488 (1 gen) are identical]. To produce urediniospores representing the race mix for first generation (for the final study), freshly produced individual races from the first generation were mixed again in equal proportion before inoculation, to obtain 4 mg of race mix inoculum.

8.2.4 FINAL INOCULATION OF THE RACES AND RACEMIXES : Urediniospores (4 mg) of the isolates (representing 1, 6 and 11 generations from each race and the racemix) were deposited on leaf disks of cvs I-488, T-173 (both 15 replications) (Fig. 8.1), and differential cultivars (5 replications each) and on some coverglasses, in a spore settling tower (section 2.4.2). The tower was thoroughly washed with 70% ethanol and blown dry, between successive inoculations. Uniformity of deposition (variation less than 5%) and of germination of the urediniospores (all > 93%) were assessed on the coverglasses. The inoculated leaf disks were placed on foam soaked with GA (10mg/L) and incubated in control growth cabinets under 'standard environment' (section 2.5.1).

8.2.5 OBSERVATIONS ON TRAITS OF DISEASE EXPRESSION: The following traits of aggressiveness (section 2.6.2) were recorded on leaf disks (separately for cvs I-488 and T-173), latent period to production of first uredinium (**LP1**), uredinia produced per leaf disk (**ULD**) and urediniospores produced per square mm of leaf area (**USM**). Shorter LP1 and higher ULD and USM reflect higher aggressiveness. Although other traits viz. IPF and LP50, were also recorded, for brevity, they are not presented here. On the leaf disks of differential cultivars, the Infection Type (IT) on a scale of 0-4 (increasing disease severity) was recorded (Appendix 7)

8.2.6 STATISTICAL ANALYSIS: The data on aggressiveness traits were examined for homoscedasticity and normality of error variance (section 2.7.1) and were appropriately transformed. The analysis of variance was performed on the factorial design (4 races x 3 generations X 2

cultivars). The cell means (15 replications) were tested for significance in differences by least significant difference (LSD) method using a two-tailed '**t**' test, employing the standard errors of difference from the second order interaction component in the ANOVA.

The significance of difference between cell means was assessed at $P = 0.05$, and this is not recurringly mentioned in the results; thus when cell means are referred to as significant, it is at $P < 0.05$, and when not significant it is $P > 0.05$.

8.3 RESULTS

8.3.1 ANALYSIS OF VARIANCE : For all traits of aggressiveness, the main effects of race, generation and cultivar were significant ($P < 0.001$) in partitioning the variation observed (except generation for ULD, $P < 0.025$) (Table 8.1). For all the traits, the proportional contribution to the variation was highest by cultivar followed by race and generation.

While most first order interactions of these three main effects were usually significant ($P < 0.001$) contributors to variation in the traits, those of generation X cultivar was not significant in explaining the variation in ULD (Table 8.1). However, the second order interaction of race X generation X cultivar was significant for all traits (Table 8.1). When the variance of the interaction effects were added to that of the residual, the level of significance of the main effects remained unchanged and thus can be discussed independently.

The significant race X cultivar interaction (generation effect held constant) for all traits of aggressiveness, indicates a degree of physiologic specialisation or specificity by these races on these two cultivars for the traits recorded. The significant race X generation interaction (cultivar effect held constant) observed for all traits suggests that the rate of disease expresssion (LP1) and of disease severity (ULD and USM) differed within atleast some of the races over generations and there was a difference in either direction and/or the level of change between the two races. Similarly the significant

Table 8.1 Analysis of variance[a] of the races and racemixture of M. medusae for three aggressiveness traits[b] representing three generations (1, 6 and 11) on cvs I-488 and T-173.

Source	D. F.	LP1	ULD[c]	USM[c]
Race (R)	3	30.81	77.39	36.76
Generation (G)	2	4.95	2.71*	4.20
Cultivar (C)	1	546.42	270.77	273.66
R X G	6	5.42	5.09	17.28
R X C	3	27.31	134.72	64.43
G X C	2	22.92	0.96ns	3.86
R X G X C	6	7.28	5.22	19.15
Residual	336	0.53	0.60	0.18[d]
Total	357	3.18	3.30	3.14[d]

[a] Mean sum of squares has been presented. All unmarked values are significant ($P < 0.001$); * denotes significant with $P < 0.025$; 'ns' denotes not significant.

[b] The three aggressiveness traits are latent period to production of first uredinium (LP1), uredinia produced per leaf disk (ULD; square root transformed) and urediniospores produced per square mm of leaf (USM; $Log_e + 1$ transformed).

[c] Using transformed values.

[d] The residual and total degrees of freedom for USM is 240 and 261 respectively.

8.2 Means[a] for three aggressiveness traits on cvs I-488 and T-173 by virulent race 5M of M. medusae cultured cv. I-488 (5M-I488) and on cv. T-173 (5M-T173), assessed after 1, 6 and 11 generations of serial culture. The values sharing the same alphabet within a row do not differ significantly ($P < 0.05$). For comparisons of values across rows, use Least Significant Difference (LSD) values indicated under each trait.

a) **LATENT PERIOD (DAYS) TO PRODUCTION OF FIRST UREDINIUM (LP1)**

	REACTION ON cv. I-488			REACTION ON cv. T-173		
GENERATIONS	1	6	11	1	6	11
RACES						
5M-I488	7.27^{a}	7.00^{a}	7.27^{a}	9.13^{b}	9.87^{c}	10.13^{d}
5M-T173	7.27^{a}	8.00^{b}	7.93^{b}	9.13^{c}	9.42^{c}	9.40^{c}

Least significant difference $(LSD)_{0.05} = 0.523$

b) **UREDINIA PRODUCED PER LEAF DISK (ULD)** (using square root transformed values)

	REACTION ON cv. I-488			REACTION ON cv. T-173		
GENERATIONS	1	6	11	1	6	11
RACES						
5M-I488	4.97^{b}	5.88^{a}	5.99^{a}	4.98^{b}	4.89^{b}	5.00^{b}
5M-T173	4.97^{a}	4.30^{b}	4.81^{a}	4.98^{a}	4.99^{a}	5.25^{a}

Least significant difference $(LSD)_{0.05} = 0.560$

c) **UREDINIOSPORES PRODUCED PER SQUARE MM OF LEAF (USM)** (using $Log_e + 1$ transformed values)

	REACTION ON cv. I-488			REACTION ON cv. T-173		
GENERATIONS	1	6	11	1	6	11
RACES						
5M-I488	6.59^{bc}	7.06^{a}	6.86^{a}	6.43^{c}	6.02^{d}	5.65^{e}
5M-T173	6.59^{a}	6.53^{a}	6.10^{b}	6.43^{a}	6.27^{a}	6.48^{a}

Least significant difference $(LSD)_{0.05} = 0.346$.
[a] mean of fifteeen replicates.

generation X cultivar interaction suggests differences in the direction or level of changes over generations on the cultivars. The significant second order interaction (race X generation X cultivar) implies that at least some of the specific two - factor interactions for any two factors differed, depending on the level of the third factor. Thus, the interaction between any two factors need to be studied separately for each level of the third factor. Hence, subsequent analysis is directed towards identifying for each race, trends across generations for different cultivars, separately for both experiments.

8.3.2. DISASSOCIATION OF AGGRESSIVENESS TRENDS IN RACE 5M ON CULTIVARS I-488 AND T-173.

The mean values for the three disease traits of 1, 6 and 11 generations on cvs I-488 and T-173 by isolates of races 5M-I488 and 5M-T173, are presented in Table 8.2.

8.3.2.1 LATENT PERIOD TO PRODUCTION OF FIRST UREDINIUM (LP1) : The aggressiveness of race 5M-I488 (race 5M cultured on cv. I-488) increased (decrease in latent period) over generations of culture (although not significantly) on cv. I-488, while on cv. T-173, it significantly decreased (increase in latent period) (Table 8.2a). The race 5M-T173 (race 5M cultured on cv. T-173) significantly decreased its aggressiveness for LP1 on cv I-488, while on cv. T-173, the changes were not significant (Table 8.2a).

8.3.2.2 UREDINIA PRODUCED PER LEAF DISK (ULD) : On the basis of ULD, the aggressiveness of race 5M-I488 increased significantly on cv. I-488, with increase in number of generations of culture, while on cv.T-173 it remained relatively unchanged (Table 8.2b) The race 5M-T173 increased its aggressiveness on cv. T-173 (but not significantly) over generations, but on cv. I-488, a decrease in aggressiveness from 1 to 6 generations was apparent, although there was no significant difference between 1 and 11 generations (Table 8.2b)

Table 8.3 Means[a] for three aggressiveness traits by race 5A and race mix 5A + 5M of *M. medusae* on cvs I-488 and T-173, assessed after 1, 6 and 11 generations of culturing on cv. I-488. The values sharing the same alphabet within a row do not differ significantly ($P < 0.05$). Use appropriate LSD values from Table 8.3 for comparisons across rows.

a) **LATENT PERIOD TO PRODUCTION OF FIRST UREDINIUM (LP1)**

	REACTION ON cv. I-488			REACTION ON cv. T-173		
GENERATIONS	1	6	11	1	6	11
RACES						
5A	8.27^{b}	7.47^{a}	7.73^{a}	-	13.15^{d}	10.36^{c}
5A + 5M	8.20^{a}	7.73^{a}	8.06^{a}	10.06^{b}	10.87^{c}	11.40^{d}

b) **UREDINIA PRODUCED PER LEAF DISK (ULD)** (using square root transformed values)

	REACTION ON cv. I-488			REACTION ON cv. T-173		
GENERATIONS	1	6	11	1	6	11
RACES						
5A	6.36^{b}	6.95^{a}	6.25^{b}	0.00^{d}	1.44^{c}	1.43^{c}
5A + 5M	4.19^{a}	3.75^{a}	3.94^{a}	2.95^{b}	2.50^{bc}	2.14^{b}

c) **UREDINIOSPORES PRODUCED PER SQ. MM OF LEAF (USM)** (using $\log_e+1$ transformed values)

	REACTION ON cv. I-488			REACTION ON cv. T-173		
GENERATIONS	1	6	11	1	6	11
RACES						
5A	7.45^{a}	7.53^{a}	7.09^{b}	0.00^{d}	4.25^{c}	4.60^{c}
5A + 5M	6.44^{a}	6.13^{a}	6.23^{a}	5.05^{b}	3.54^{c}	2.94^{d}

[a] mean of fifteen replicates.

8.3.2.3 UREDINIOSPORES PRODUCED PER SQUARE MM (USM) : The race 5M-I488 significantly increased its aggressiveness on cv. I-488 with increasing generations of serial culture, but on cv. T-173 it decreased significantly (Table 8.2c). The aggressiveness of race 5M-T173, however, significantly decreased on cv. I-488, from 1 to 11 generations, but on cv. T-173 it remained relatively unchanged from 1 to 11 generations (Table 8.2c).

8.3.3. SURVIVAL OF VIRULENT RACE IN MIXTURE WITH THE AVIRULENT RACE

The aggressiveness (three traits) of the racemixture and race 5A, over the course of serial culture (1, 6 and 11 generations), on cvs. I-488 and cv. T-173 are presented in Table 8.3.

8.3.3.1 LATENT PERIOD TO PRODUCTION OF FIRST UREDINIUM (LP1) : The avirulent race 5A increased its aggressiveness on cv. I-488 for LP1 with an increase in number of generations (Table 8.3a). However, in contrast to those observed in an earlier study (Chapter 6), race 5A exhibited slight virulence on cv. T-173 with increase in number of generations, but with delayed appearance of uredinia (Table 8.3a). The aggressiveness of the racemix (5A +5M) increased on cv. I-488 (not significantly) with increase in number of generations of culture, but on cv. T-173, there was a decrease in aggressiveness (Table 8.3a).

8.3.3.2 UREDINIA PRODUCED PER LEAF DISK (ULD) : The aggressiveness for ULD by race 5A, increased from 1 to 6 generations, but between 1 and 11 generations there was no significant difference (Table 8.3b). On cv. T-173, race 5A produced a few small uredinia at generation 6 and remained relatively unchanged at generation 11. Although, the aggressiveness of the racemix on cv. I-488 decreased after generation 1, the change was not significant. However, the racemix, compared to both races 5A (Table 8.3b) and 5M (Table 8.2b), had considerably reduced aggressiveness on this cultivar, suggesting some antagonistic interactions between the virulent and avirulent races when mixed. On cv. T-173, the racemix had significantly reduced aggressiveness over serial culture (significant between 1 and 11 generations) (Table 8.3b). When backtransformed values were considered (cf. Table 8.2b), the aggressiveness of racemix on cv.

T-173 was always less than half of those observed by race 5M on this cultivar, indicating the interference by race 5A component of the mixture on the aggressiveness of race 5M on this cultivar.

8.3.3.3 UREDINIOSPORES PRODUCED PER SQUARE MM (ULD) : The race 5A had relatively reduced aggressiveness between 6 and 11 generations on cv. I-488, while on cv. T-173, the changes were not significant between 6 and 11 generations (Table 8.3c). The racemix (5A + 5M) although reduced its aggressiveness over generations on cv. I-488, such reduction was not significant (Table 8.3c). On cv. T-173, the racemix consistently and significantly exhibited decrease in aggressiveness from 1 to 6 and 6 to 11 generations. As observed for ULD, the aggressiveness of the racemix for USM was always less on cv. I-488 compared to the mean sum of aggressiveness of races 5A and 5M on this cultivar (cf. Tables 8.2c and 8.3c). Similarly, the aggressiveness of the racemix on cv. T-173 was always less than half of the aggressiveness of race 5M on this cultivar (using back transformed values from Tables 8.2c and 8.3c). Such differences were more pronounced when back transformed values were considered.

A generally similar results were obtained in a similar study employing race 4B and the natural virulent mutant race 4M (Appendix 7).

8.3.4 CHANGES IN THE REACTION OF THE RACES ACROSS GENERATIONS ON A RANGE OF POPLAR CULTIVARS :

Both the races and racemixes, representing three generations (1, 6 and 11) of serial culture, were inoculated on leaf disks of twelve cultivars of poplars in each experiment. Changes in the reaction of the races (cultured on cv. I-488 or T-173) were assessed on a simple scale of 0 - 4 infection type (IT) (section 2. 6) in both experiments (8.1 and 8.2) and the data are presented in the Appendix 7. Although the aggressiveness traits like LP1 and ULD were recorded on these cultivars, they are not presented here in an effort to simplify the presentation; but rankings of the isolates based on infection type were consistent with that for LP1 and ULD.

In both experiments (i.e. involving radiation and natural virulent mutant), over generations, there were frequent consipicuous trends in the infection type (IT) of most races on most cultivars (Appendix 7). The cultivars from each experiment could be grouped into one of the following five classes 1) those associated or correlated with observed general changes of aggressiveness on cv. I-488, over generations (cv. W-79/304), 2) those associated with the response of cv. T-173 (cv. W-79/306), 3) those on which aggressiveness increased irrespective of the race or on the cultivar on which the races were cultured (cvs I-154, 60/164, 82-PH-1 and 65/70), 4) those on which aggressiveness always decreased (7-2 and 60/166), and 5) those cultivars on which there were no observable changes in IT over generations or the changes were inconsistent (cvs. 10-3 and 40-2).

Although certain cultivars were in the same class in both experiments (or were included in only one experiment), the distinction of others was not consistent between experiments. For example, the response of cv. Hickeliana to races, was similar to that of cv. T-173 in Expt. 8.1, but no change was observed on this cultivar in Expt. 8.2 (Appendix 7). Such contrasts probably reflect the differences in the races in their ability to respond to host selection pressure.

On these cultivars, the wild types exhibited the narrowest virulence spectrum, followed by the racemixes, while the mutants had the widest virulence spectrum, in both experiments. The reaction of the wild type and the mutants corroborate with the results of an earlier study (Chapter 5).

8.4 DISCUSSION

This study examined the changes in aggressiveness (various traits) by virulent and avirulent races of M. medusae when cultured on two cultivars of poplars, for possible occurrence of disassociation of the aggressiveness in, and assessed the survivability of, the virulent race in mixture with an avirulent race on the susceptible cultivar over generations. Although the results of studies with race 5M and 5A are discussed here, those employing races 4M and 4B were essentially similar (Appendix 7).

The cv. I-488 was usually more susceptible to all the isolates than cv. T-173. On cv. I-488, the relative aggressiveness of the virulent mutants, when compared to their respective wild types, was low for traits of disease severity (ULD and USM), but high for latent period. These observations are consistent with that of the earlier study (Chapter 6) and thus, the relative level of aggressiveness in both races and of the susceptibility of both cultivars are not experimental artefacts. The directional changes in the aggressiveness of the race 5M on cv. I-488 also parallel with the results of previous study (Chapter 7).

8.4.1 DISASSOCIATION OF THE AGGRESSIVENESS TRENDS IN THE VIRULENT RACE : Race 5M, when cultured on either of the two cultivars, exhibited an increase in the aggressiveness for some traits, towards it's 'own' cultivar, accompanied with reduction in the aggressiveness on the 'other' cultivar. When cultured on cv. I-488, race 5M developed higher aggressiveness (shorter LP1, higher ULD and USM) on this cultivar at the end of 11 generations compared to that of the initial population (1 gen). However, it had reduced aggressiveness when tested on the other cultivar, T-173 (Longer LP1 and smaller USM). Also, despite an increase in the aggressiveness for traits of disease severity (ULD and USM) by race 5M on cv. I-488 at the end of 11 generations, its mean aggressiveness was always lower than that of the race 5A at that specific generation. Further, on cv. I-488, the aggressiveness of race 5M-I488 did not increase between 6 and 11 generations. This suggests that, on this cultivar, the race 5M may have reached its maximum parasitic potential by 6 generations, and further increase was not possible, which may be due to the inherent virulence load it possesses (Leonard, 1977). Again, this observation is consistent with an earlier interpretation (Chapter 6) that aggressiveness potential of a race on a susceptible cultivar may be dependent on the a/virulence make up of the race, and 'unnecessary' virulence may limit the aggressiveness of the races on a susceptible cultivar.

However, on cv. T-173 such directional selection for increased aggressiveness was observed only for the trait ULD. Thus the resistance (quantitative) of this cultivar, as measured by latent period and

sporulation, to this race appears to be fairly stable.

The reaction of the differential cultivars to the races representing increasing generations of serial culture (Appendix 7), is further evidence of the disassociation of aggressiveness trends in these two races. Those cultivars which fell consistently into certain classes may be of significance in creating heterogeneity by mosaic or mixture of such cultivars. Those cultivar groups on which the aggressiveness of the pathogen tends to disassociate among themselves (e.g. the I-488 group and T-173 group) along with those on which aggressiveness tends always to decrease (cvs 7-2 and 60/166) (Appendix 7), are worthy of further trials in the field to test results from laboratory studies.

The cvs 10-3 and 40-2, which remained consistenly resistant to all the isolates of the races over generations (Appendix 7), are also of interest for further field testing. However, some uredinia developed on both these cultivars in previous experiments (Chapter 5) but such infections were not observed in the present study. This may be due to one of the following :

1) The resistance genes of these two cultivars may be 'strong' (sensu Vanderplank, 1968, 1982), thus the corresponding virulence allele in the pathogen has low fitness to survive in the absence of their specific resistance gene.

2) The background genes that determine the fitness of the present races may not match the host genotypes of these two cultivars and thus although virulence towards these cultivars has been observed earlier, it is not sustained for lack of fitness traits.

8.4.2. THE INTERACTION OF VIRULENT AND AVIRULENT RACES ON CV. I-488 : The racemixture representing generation 1 were obtained by physically mixing, in equal proportions, of races 5A and 5M. Thus, in the absence of synergistic or antagonistic interaction between these two races on a susceptible cultivar (I-488), the mean aggressiveness of the racemix (1 gen) should approach that of the mean of the sum of individual races in the same generation. For all traits, but more pronouncedly in traits of disease severity, there was evidence of antagonistic interaction of the races resulting in a reduced level of disease. As the virulent and

avirulent races are expected to have a common genetic background, it is tempting to suggest that such a reaction results mainly from the interaction of a/virulence products.

Aggressiveness of the racemix on cv. I-488 did not change appreciably with the serial culture and in some instances it decreased, with increasing number of generations. This contrasts with the behaviour of the pure races, (race 5M cultured on cv. I-488, Table 8.2; races 4B and 8A, Chapter 7) which exhibited increasing aggressiveness over generations. Thus the low aggressiveness and absence of strong directional trend of increase in aggressiveness in the racemixtures on the susceptible cultivar, indicate that, atleast within the experimental system, individual races are fitter to survive than the mixtures of virulent and avirulent races. This observation is supported also by the lack of marked increase in the aggressiveness of the wild types on cv. I-488 over generations (in contrast to results of Chapter 7), as in this experiment the wild type race 5A exhibited some virulent mutant biotypes in the population (Table 8.2b), which effectively makes the population a mixture of races. These observations have significant implications in disease control as the diversity in the host genotypes in cultivar mixtures may lead to racial polymorphism of the pathogen population. This variability may result in less disease due to the interactions of races or biotypes, not only on the susceptible cultivar but also on the resistant cultivar due to the factors of induced resistance (see Chapter 11). This possible reduction in disease level would be additional to that suggested to result from other factors (Chapter 10). Chin _et al_ (1984) made similar observations with _Erysiphe graminis_ DC f. sp. _hordei_ Marchal on Barley, where a mixture of or sequential inoculations of virulent and avirulent isolates in either order, always resulted in lower conidial production than that of virulent isolate alone on the resistant cultivar. A decrease in the aggressiveness when races of _M. medusae_, other than those employed here, were mixed in differing proportions and inoculated on cv. I-488 has been observed (Prakash, _unpublished_). Rayner (1983) also observed apparent antagonism between two races of _M. larici-populina_ when inoculated together on susceptible cultivars of poplar (single monocycle) and concluded that extent of such an interaction was dependent on the proportion of the component races in the mix. Antagonism between races have also been observed by Watson

(1942) with P. graminis f. sp. tritici of wheat, and by Thurston (1961) with Phytopthora infestans of potato.

8.4.3 SURVIVAL OF THE VIRULENT RACE IN MIXTURE WITH AN AVIRULENT RACE : In the racemixture, the race 5M initially constituted only half of the total population and thus the aggressiveness of the racemix as observed by ULD and USM on the cv. T-173 is attributable to the proportion of this race in the racemixture population. At the end of eleven generations, the aggressiveness of the racemix on cv. T-173 reduced considerably, characterised by slower rate of disease progress and reduced disease severity; such reduction in disease severity was more pronounced for sporulation (USM) than the uredinial number (ULD). Thus the proportion of, and aggressiveness of, race 5M was reduced when it was cultured, on a susceptible cultivar, in mixture with race 5A. This indicates that a race with a relatively narrow virulence spectrum may be fitter to survive on susceptible cultivars in competition with a race with wider virulence spectrum. However, despite the higher aggressiveness of race 5A on the susceptible cultivar, the virulent races were not completely eliminated from the mixtures, although such a phenomena where one race completely displaces the other, have been observed in few racemixture studies (Brown, 1975). Further, the proportion of virulent mutants in the wild type population (5A) cultured on cv. I-488 increased slightly over generations. This inconsistency may stem from either or both of the following reasons:

1. The shorter latent period of the virulent mutants compared to the avirulent wild type on cv. I-488 may confer a degree of fitness which in a single discrete inoculation method as employed in this study can compensate for the lower infection efficiency and sporulation, as virulent races (mutants) erupt a day or two earlier.

2. If the mutation for virulence occurs a in super-aggressive biotype (higher than the mean of the avirulent population), then it still would have considerable fitness despite its cost of virulence.

If only the first reason is considered, then the frequency of virulent

allele in the natural avirulent population in field on a susceptible cultivar would be far less than observed here, because in a polycyclic epidemic the shorter latent period by one or two days of the virulent mutants may be insufficient to compensate for the reduction in infection efficiency and sporulation because of the continuity of the inoculations that would occur as opposed to single 'one-time' inoculations in the present study (K. J. Leonard, pers. commun.). Also, the above two reasons may account for the appearance and maintenance of the mutant biotypes in race 5A (and also race 4B; Chapter 6 and Appendix 7) which are virulent towards cv. T-173. In the racemix, the lack of increase in the frequency of virulent races due to new mutations such as those observed in the wild type races, are difficult to explain in simple terms.

In the first generation isolate of the racemix, where race 5M constitutes half the population, considerable reduction in aggressiveness was evident on cv. T-173 compared to the reaction of the individual race 5M. For example, on cv. T-173, racemix (1 gen) had decreased aggressiveness for latent period (increased LP1) when compared to race 5M; similar observation was made for ULD. Such deviations suggest interferences by the avirulent race in functioning of the virulent on the resistant cultivar, and this may also have epidemiological implications.

8.4.4 GENERAL IMPLICATIONS OF THE INVESTIGATIONS : Despite the directional selection of aggressiveness in races due to observed plasticity of the quantitative traits, the occurrence of the disassociation of the aggressiveness trends in the virulent races on the cultivars of poplar may have practical implications in disease control through deployment of cultivar mixtures. Similar disassociation of aggressiveness trends has been observed in other system (Triantaphyllou,1975; Hiura, 1978; Wolfe et al, 1983; Alexander et al, 1984a).

When two or more cultivars, which do not support the uni-directional selection for enhanced aggressiveness, are planted in mixtures, although a race virulent on all the cultivars could arise, such a race upon infecting a cultivar would be expected to progressively

increase its aggressiveness on this cultivar, but may gradually lose its aggressiveness on the other cultivars. Such a system would pose an evolutionary dilemma for the pathogen (Wolfe, 1984) and thus, although individual selection by host genotype would be for high aggressive races, group selection might ensure moderate aggressiveness in the pathogen population (Parlevliet, 1983a). The present results also suggest that on certain cultivar combinations favour positive association of aggressiveness trends in these races (Appendix 7). Thus, the cultivar mixture can be expected to be succesful as means of disease reduction only with those cultivar combinations on which the races exhibit diverging aggressiveness trends, and on which the virulence spectrum and aggressiveneness are negatively associated (e.g. cv. I-488 and T-173).

Had the serial culturing been extended for a further ten or twenty generations, the virulent races cultured on either cultivar may possibly have become very distinct and may have been recognisable as distinct races (sensu Stakman and Christensen, 1953), i.e. as gradual reduction in aggressiveness by a race on a cultivar may result eventually in the the attenuation of virulence of this race on this cultivar. Similarly, because of the apparent reduced aggressiveness of the virulent race, the proportion of the virulent race in the race mixture, over generations, may be reduced to the natural mutation frequency. Thus, although observed as aggressiveness disassociation in the present study, such trends may manifest as virulence disassociations (cf. Alexander et al, 1984b), which are apparent only in the long run. While the virulence disassociation, which may manifest due either to physical linkage of virulence and avirulence genes or selective forces (Vanderplank, 1982; Wolfe and Knott, 1982; Wolfe et al, 1983; Alexander et al, 1984), is helpful in combining those resistance genes corresponding to virulence genes from opposing groups, the aggressiveness disassociation helps to reduce the levels of disease despite the combinations of such virulence genes. Vanderplank (1982) has discussed the effect of environmental variables such as temperature on such virulence associations.

8.5 SUMMARY

When the virulent races of M. medusae were serially cultured on cv. I-488, for 11 generations, although an increase in the aggressiveness (most traits) with increasing number of generations on this cultivar was evident, there was a decrease in the aggressiveness of these isolates on cv. T-173. Similarly, the races cultured on cv. T-173, decreased in their aggressiveness on cv. I-488, although cv. T-173 did not exhibit strong selection on races towards higher aggressiveness. When the virulent and avirulent races were mixed (1:1) and serially cultured for 11 generations on cv. I-488, the aggressiveness of the racemix decreased on cv. T-173, with an increasing number of generations, suggesting the reduced survivability of the virulent race in competition with the avirulent race on the susceptible cultivar. An antagonistic interaction between the two races was observed on cv. I-488. The results emphasise the need to promote polymorphism in the pathogen by heterogeneity in the host, and to exploit specificity in the host to effect disruptive selection on the pathogen.

Section 11

SECTION II

CHAPTER 9

EFFECT OF CHANGING TEMPERATURE AND LIGHT INTENSITY ON HOST-PATHOGEN INTERACTIONS IN THE *M. MEDUSAE* - *POPULUS* SYSTEM

AN INTRODUCTION

9.1 IMPORTANCE OF THE ENVIRONMENT IN HOST-PATHOGEN SYSTEMS : The physical environment, with the host and pathogen, is a component of the disease triangle. The role of environment in host-pathogen situations is two-fold. First, the environment directly influences the level of disease by its action on the host or pathogen or both (Colhoun, 1973). Second, the environment is an evolutionary force acting on the host-pathogen systems, and thus may be significant in maintaining the delicate host-pathogen balance (Heather and Chandrashekar, 1982).

Many studies have been conducted to assess the direct influence of environmental variables independently on the pathogen, or on the host, and on disease expression in the pathosystems of agricultural crops (Walker, 1965; Colhoun, 1973), and in leaf rusts of poplars (Heather and Chandrashekar, 1982; Singh and Heather, 1982 a & b and 1983). However the evolutionary importance of the environment in influencing host-pathogen interactions has been less studied.

Many variables of the physical environment viz. temperature, light intensity, photoperiod, humidity, etc. would be interacting with the host-pathogen systems. However, temperature is probably the most important of these variables and thus it's role is relatively better understood than that of other factors (Vanderplank, 1978 and 1982). In the following review, only few examples, relevant to the objectives of this Section (II), are presented. Due to the abundance of reports of the effects of temperature rather than those of other variables, this literature is cited more frequently.

9.2 EFFECT OF ENVIRONMENT ON HOST RESISTANCE : Influence of environment on the qualitative resistance of plant hosts to pathogens has long been recognised (e.g. Gassner and Straib, 1934; Newton and Johnson, 1936). Both pre-inoculation and post-inoculation temperatures could affect the resistance patterns. For example, Gassner and Straib (1934) noted that pre-inoculation temperature below 14°C often increased susceptibility in wheat to stripe rust [Puccinia glumarum (Schm.) Erriks. and Henn.]. Similarly, wheat plants susceptible to stripe rust at 12.9°C became resistant when exposed to a temperature of 25.3°C (Newton and Johnson, 1936). Dyck and Johnson (1984) found many lines of wheat with single genes for resistance to Puccinia recondita Rob. ex. Desm., to be highly sensitive to post-inoculation temperature, but the reaction of some lines was relatively more independent of the changes in temperature. Likewise, Brown and Sharp (1969) have reported temperature sensitivity of minor genes of resistance in wheat to P. graminis f. sp. tritici.

Studies on resistance of poplar clones to M. larici-populina (Heather and Chandrashekar, 1982) and M. medusae; Singh and Heather, 1982b) have clearly established the high environmental lability of these systems. Both studies revealed that some clones of the poplar were highly susceptible to certain races of the leaf rust when incubated at particular combinations of temperature (pre- and post-inoculation) and light intensity, but less susceptible at other combinations.

The effect of temperature on resistance can be qualitative, characterised by clear cut changes from resistance to susceptibility or vice versa. It can also be quantitative, where such changes are manifested in the degree of susceptibility for various disease traits. Singh and Heather (1982b) observed that certain clones of poplars were highly resistant to certain races of M. medusae when incubated at 25°, but susceptible at 15°C, while some other cultivars showed the opposite trend. Similarly cultivars of wheat containing Sr6 gene for resistance to certain races of P. graminis f. sp. tritici were completely resistant at 15° but susceptible at 27°C (Watson and Luig, 1968b). Similar changes in qualitative resistance in other systems have been reported by Green and Johnson (1955), Chandrashekar and Heather (1981) and Dyck and Johnson (1984).

The effect of environment on quantitative changes in resistance can be assessed (in rusts) by changes in the latent period, infectious period, uredinial number, sporulation, disease progress etc. Generally it has been observed that increasing temperature results in decreasing latent period (Teng and Close, 1978; Eversemeyer et al, 1980; Singh and Heather, 1983). Singh and Heather (1983) observed a decrease in resistance of the poplar to M. medusae with increasing light intensity of incubation, and also observed changes in the latent period, uredinial number and sporulation as affected by interaction of the temperature, light intensity and photoperiod.

While a few studies have shown that with an increase in temperature of incubation the resistance of the host decreases, a reverse trend has been observed in many studies. Following an extensive review of some of the work which supported the former trend, Vanderplank (1978) hypothesised that " if temperature affects resistance, it affects it in the direction of reduced resistance at higher temperature." He suggested that, in instances where the results show otherwise (i.e. increase in resistance with increase in temperature), the temperature levels employed to obtain such resistances were above the cardinal limit for the development of the pathogen. Ellingboe (1978) has also implied that such trends of increasing resistance with decreasing temperature are a universal phenomena.

However, many recent studies have shown that the higher temperatures which elicit resistant reactions in hosts, were within the upper cardinal limit for the system. For example, Hyde (1982) reported that wheat cv. Maris Fundin was resistant at 20°, partially resistant at 15° and susceptible at 9°C, to P. recondita. The limits of temperature and light intensity adopted in studies of Heather and Chandrashekar (1982) and Singh and Heather (1982 and 1983) were also within the cardinal limits of the respective pathogens studied and were similar to the environmental range observed naturally in the poplar growing regions of Australia. Dyck and Johnson (1984) referring to Vanderplank's (1978) hypothesis, also showed many exceptions to his theory in the response of Lr genes in wheat to P. recondita.

9.3 SPECIFICITY AND PLASTICITY OF PLANT PATHOGENS TO THE PHYSICAL ENVIRONMENT : While some of the works cited earlier (section 9.2) dealing with the role of environment on host resistance included the interaction of the pathogen also, there are many studies which have demonstrated differences in the preference of the pathogen in their virulence and aggressiveness to certain environmental regimes, on occassions independent of the host effect. Waggoner and Wallin (1952) used the term 'environmental races' to describe cultures which respond differently to varying environments while other workers have used the term 'ecotypes' (Boyd et al, 1969) or 'ecological races' (Line and Bugbee, 1964; Nelson, 1979). There are similar instances of specificity of certain pathogen cultures to certain environments in a few pathogens (Shepherd and Pratt, 1973; Leonard, 1977a). Bisby (1943) was possibly the first to implicate the role of environment as a critical factor in influencing the fungal distribution. Effect of the physical environments on pathogen, as on host resistance, can be qualitative or quantitative. Qualitative effects include changes in a/virulence, while quantitative effects are on changes in aggressiveness traits e.g., latent period, uredinial production and sporulation. Singh (1983) found some races of M. medusae being more aggressive on certain clones of poplar at certain temperature and light intensity combinations than at others. Chandrashekar (1981) made similar observations on aggressiveness of races of M. larici-populina. Sood and Sackston (1972) reported that the optimal temperature for penetration of sunflower by Puccinia helianthi Schw. varied among strains; while some strains penetrated best at 15-20°, others penetrated well at 20-25°C. They suggested that those strains having the widest temperature optima were likely to have a survival advantage over those with a narrow range. Cassel (1939) made similar conclusions on germination of races of P. graminis f. sp. tritici.

There are a few instances where certain pathogen cultures show preference to their 'own' envrironment, i.e. the environment from which they were isolated. For example, Boyd et al (1969) found Western Australian cultures of many barley pathogens had consistently lower temperature optima compared with cultures from USA or Europe. This corresponds with the temperatures during the growing seasons in these

locations. Browning (cited in Brown, 1975) isolated a strain of Puccinia coronata Corda from high altitudes in Columbia that had a lower temperature optima than North American isolates, reflecting the lower temperatures expected to prevail in such high altitudes. Similarly, Shepherd and Pratt (1973) found some isolates of Phytopthora drechsleri Turner from warmer regions to have a higher temperature optima compared to those from cooler regions. McCoy and Blakeman (1976) observed some isolates of Mycosphaerella ligulicola Baker, collected from the glasshouse were relatively more tolerant to darkness while those collected from the field were more tolerant to UV light.

These observations clearly suggest that, adaptations may have occurred in these pathogens to suit certain ecological conditions prevailing in the specific area. Thus, the environment may have acted as an agent in selecting those biotypes which are fittest to survive in a specific niche. Such adaptations, or even lack of them, would have practical epidemiological implications to shifts in populations, pathogen evolution and prediction of potential disease levels. For example, Katsuya and Green (1967) showed that temperature specificities of races of P. graminis f. sp. tritici may determine the ability of the races to survive when mixed. They suggested that the rise to predominance of race 56 during 1956-61 in Canada may be related to the aggressiveness of that race in moderate to warm seasons which prevailed during that period i.e. the conditions were more suitable for this race. Nelson (1979) also suggested that ecological specificity of race T of Helminthosporium maydis was a critical factor in preventing the pathogen from reaching epidemic proportions during 1971 in the midwestern corn belt of USA, while Hill and Nelson (1976) provided some experimental evidence to support this theory.

Heather and Chandrashekar (1982), based on their results which showed the high environmental lability of the poplar-leaf rust system, concluded that environment may be an important factor in maintaining the diversity of pathogen populations as a consequence of differential selection pressures on the host-pathogen combinations and hence result in stability of the pathosystem. However there is a dearth of studies which have investigated experimentally the plasticity of the pathogen in

its ability to respond by population changes to environmental selection pressure, or indeed if environmental heterogeneity can promote polymorphism amongst pathogens. Line and Bugbee (1964) demonstrated that some cultures of P. graminis f. sp. tritici could adapt in their germination to cold temperature (3-5°C) after selection for 20 cycles at this temperature, but such isolates lost their germinating ability at normal temperatures.

9.4 USE OF ENVIRONMENTAL SENSITIVITY IN BASIC STUDIES ON THE HOST-PATHOGEN RELATIONSHIP : Temperature sensitivity of the host or pathogen for disease reaction is ideal for the study of the biology of host-pathogen relationship. Such sensitivity provides makes possible comparative biochemical or physiological assays of resistance or susceptibility in the host, and virulence or avirulence in the pathogen without changing the genotypes of the host or pathogen involved.

Daly (1972) used the Sr6 gene in wheat for resistance against P. graminis f. sp. tritici race 56 to analyse many quantitative, chronological and biochemical aspects of resistance. The resistant reaction of the dominant Sr6 allele is temperature sensitive, resulting in resistance in the cultivar when incubated at 20° but susceptibility when incubated at 24-25°C. By reciprocal transfers from high or low temperatures at various times after inoculation, Seevers and Daly (1970) found that compatibility or incompatibility is determined between 60-80 h after inoculation. Jones and Deverall (1977) observed that in wheat cultivars with the Lr20 gene for resistance against a culture of P. recondita, the temperature sensitive interaction resides in the host and they concluded that resistance seemed to be associated with changes in host cells leading to wide apread collapse of the protoplast.

Ellingboe (1977) has advocated the use of temperature sensitive mutants in the pathogen, similar to those used in other microorganisms such as Neurospora or yeasts, to study the aspects of basic compatibility in plant pathogens and to isolate the gene products necessary for the positive function of parasitism. Gabriel et al (1979), after obtaining temperature sensitive mutants of Phyllostica maydis that were unable to grow at high temperatures on the host, but which grew on agar media at this temperature, suggested that host-

parasite specificity is for incompatibility, because mutations increasing virulence were concluded to be due to losses rather than gains in function. They also proposed that patterns of temperature sensitivity of certain mutants indicate that avirulence may be one pleiotropic function of genes which otherwise supports the basic life processes of the organism. Vanderplank (1978, 1982) has discussed some biochemical and evolutionary implications of the patterns of temperature sensitivity observed in various pathosystems.

CHAPTER 10

SELECTION PRESSURE BY INCREASING TEMPERATURE AND LIGHT INTENSITIES ON *MELAMPSORA MEDUSAE*

10.1 INTRODUCTION

The ability of plant pathogenic fungi to expand their spectrum of virulence (Day, 1974; Watson and Luig, 1968a) and their aggressiveness (Caten, 1974; Leonard, 1984) on host genotypes has been considered (Section I). Similar adaptation by pathogens to changes in elements of the physical environment, could explain the ecological plasticity of certain plant pathogenic fungi, which have been observed to adapt to new niches e.g. beyond their normal seasonal or geographic occurrence (e.g. Martin, 1949). While environmental sensitivity in the resistance of hosts to pathogens has been well studied (e.g. Vanderplank, 1978, Heather and Chandrashekar, 1982; Dyck and Johnson, 1984), similar sensitivity in the virulence and aggressiveness of pathogens has been less studied although considerable shifts in racial composition of some pathogens have been attributed to their environmental specificities (Katsuya and Green, 1967; Nelson, 1979).

Race 4A of *M. medusae* is compatible (+) [Infection Type (IT) : 3-4] with *P. deltoides* cv. W-79/307 when incubated (post-inoculation) at low temperature and light intensity (17°C/100 uEm^{-2}s^{-1}), but almost incompatible (-) (IT: 1-2) at high temperature (26°C), and fully incompatible (-) (IT: 1) at high light intensity (700 uEm^{-2}s^{-1}) (Table 10.1).

This chapter reports the adaptation of race 4A for compatibility with cv. W-79/307 at 26°C or 700 uEm^{-2}s^{-1} following serial culturing and selection, in separate experiments, of the host/pathogen combination in ascending temperature (Expt. 10.1), or light intensity (Expt. 10.2) regimes. The relative aggresssiveness of isolates of the race, selected at certain levels in the temperature or light intensity

series, is also reported. A summary of a similar but separate preliminary study on temperature selection has been presented elsewhere (Prakash and Heather, 1983).

Table 10.1 The compatibility (+) or incompatibility (-) of race 4A (base population) of M. medusae on P. deltoides cv. W-79/307 at different temperature and light intensity combinations of incubation.

Temperature (°C)	Light intensity (uEm-2s-1)	
	100	700
17	+	-
26	-	-

10.2 MATERIALS AND METHODS

10.2.1 PRODUCTION OF ISOLATES ADAPTED TO SPECIFIC ENVIRONMENTS : The procedure for selection of isolates adapted to specfic regimes of incubation is illustrated in Fig. 10.1. This experimental design involved serial culture of race 4A (three cycles in each regime) on detached leaves of of cv. W-79/307, [supported on gibberellic acid, in separate growth cabinets (± 1°C temperature variation, 100 uEm^{-2}s^{-1} light intensity, 16 h photoperiod)] maintained at temperatures of 17, 20, 23 or 26°C (Expt. 10.1) (Stage I of Fig. 10.1). Although, initially very few uredinia developed when isolates from 23°C regime were inoculated and incubated at 26°C, a further four generations of culture at the latter temperature enabled production of sufficient inoculum for the experiment. A similar set of isolates was obtained by serial culturing (three cycles) of race 4A on comparable leaves in a further set of growth cabinets (± 20uEm^{-2}s^{-1} variation in light intensity, 16±1°C temperature, 16 h photoperiod) held at light intensities of 100, 300, 500 or 700 uEm^{-2}s^{-1} (Expt. 10.2) (Stage I of Fig. 10.1). As in the temperature study, the isolates from 500 uEm-2s-1 produced very few uredinia initially when incubated at 700 uEm-2s-1, but upon subsequent

Figure 10.1. The sequence of inoculation followed to obtain varying temperature or light intensity selected isolates of M. medusae on detached leaves of P. deltoides cv. W-79/307. The nos. 1, 2, 3 and 4 refer to 17, 20, 23 and 26°C for temperature study (Expt. 1) and 100, 300, 500 and 700 uEm-2s-1 for light intensity study (Expt. 2). Stage I shows the sequence of serial culture at each environmental regime, and Stage II shows the final multiplication of the urediniospores of the populations selected at various temperature or light intensity for experimental inoculations. Stage III shows the final inoculations of the leaf disks of the cv. W-79/307 with the spores of the selected isolates. Sets of replicate leaf disks (15 for each urediniospore isolate) were incubated at each of the four temperature or light intensity regimes.

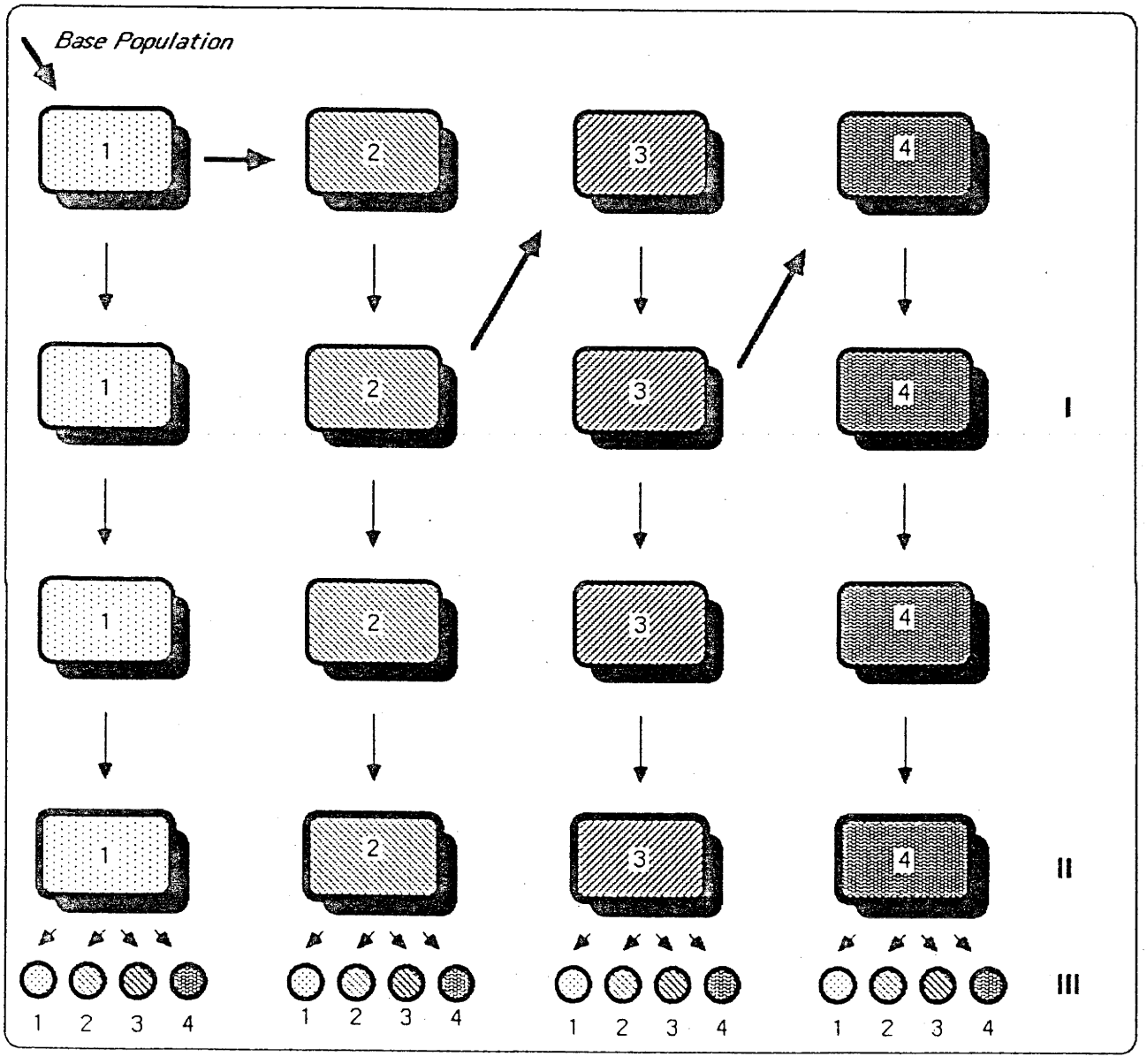


Fig. 10.1

serial culture at the latter light intensity, the isolates demonstrated comparable aggressiveness.

Hereafter these isolates are referred to as, for example the 17 T- or the 700 LI- isolate, in Expt. 10.1 and Expt. 10.2, respectively. After completion of three cycles in a particular environment, the urediniospores of the isolate were collected, dried, labelled and stored (-14° C).

When all isolates had been prepared they were removed from storage, inoculated separately on a further series of surface sterilised detached leaves of cv. W-79/307 and incubated for a single cycle at the appropriate temperature or light intensity regime, to produce fresh urediniospores of uniform age (Stage II of Fig. 10.2). These latter urediniospores were used for evaluation of virulence and aggressiveness at different temperatures (Expt. 10.1) or light intensities (Expt. 10.2) of incubation.

10.2.2 EVALUATION OF AGGRESSIVENESS OF ISOLATES SELECTED AT DIFFERENT ENVIRONMENTS : Leaf disks were punched from surface sterilised leaves of uniform age from plants of cv. W-79/307 and shaken in a sterile polythene bag to ensure randomisation and thus subsequent uniform disease expression within a treatment. The fresh, dry spores of each T- or LI - selected isolate were deposited (5 mg for Expt. 10.1 and 4 mg for Expt. 10.2) separately on replicate leaf disks (80 for Expt. 10.1, and 60 for Expt. 10.2) and on some cover glasses, in a spore settling tower (section 2.4.2). The lower spore load employed in Expt. 10.2 resulted from the decreased spore production by certain isolates in that study. The spore tower was sterilised, (washed with 70% ethanol) and blown dry between successive inoculations. For each isolate, uniformity of deposition (variation less than 5%) and germination of the urediniospores (more than 95% in all isolates) were checked on the coverglasses. Replicate leaf disks (20 in Expt. 10.1 and 15 in Expt. 10.2), were incubated in plastic Petri dishes, initially at $16\pm1^{\circ}C$ under diffused light (30-50 $uEm^{-2}s^{-1}$) for 24 h, to permit uniform, maximum spore germination, and penetration of the disks by germ tubes (Singh and Heather 1982c). Subsequently, Petri dishes were incubated at one of the

four temperatures (17, 20, 23, 26° C, constant light intensity 100 uEm-2s-1; Expt. 10. 1) or one of four light intensities (100, 300, 500, 700 uEm-2s-1, constant temperature 16°C ; Expt. 10. 2) in separate modified Phytotron L. B. cabinets (16 h photoperiod, cool, Philips® fluorescent tubes, Morse and Evans 1962) (Fig. 10.1). Hence both experiments consisted of a factorial design of four isolate populations X four environment/incubations (Stage III of Fig. 10.1).

10.2.3 OBSERVATIONS ON DISEASE EXPRESSION : Both virulence and aggressiveness were recorded for the isolates. Changes in the virulence were recorded qualitatively as Infection Type (IT), at the end of the monocycle, on a 0 - 4 scale of increasing disease severity (section 2.6.1) where 0 - immune, with no macroscopic symptoms, 1 - chlorotic/necrotic spots, 1^a - necrotic spots with few minute uredinia, and 2 to 4 - increasing number and size of uredinia. The ITs 0, 1 and 1^a represent avirulent, while 2 to 4 denote virulent, reactions.

Four traits of aggressiveness were recorded, viz. 1) incubation period (days) to flecking (**IPF**); 2) latent Period (days) to the production of the first uredinium (**LP1**); 3) number of uredinia per leaf disk (**ULD**), and 3) number of urediniospores produced per mm2 (**USM**) (section 2.6.2).

10.2.4 STATISTICAL ANALYSIS : The analysis of variance (ANOVA) was performed for the 4 X 4 factorial design using transformed values for ULD and USM, after testing the data for homoscedasticity and normality of error variances (section 2.7.1). The treatment means were compared using student's 't' test (P = 0.01) employing the standard errors of difference of the interaction component from each trait.

For all the treatments in both studies, curves of disease progress (mean number of uredinia per leaf disk were recorded daily throughout the monocycle) were fitted using the following quadratic model (Section 2.7.3).

$$y_i = b_0 + b_1 x_i + b_2 x_i^2 + e_i$$

10.3 RESULTS

10.3.1 OBSERVATIONS ON INFECTION TYPE OF ISOLATES (BOTH EXPERIMENTS) : When the population of race 4A of M. medusae was serially cultured in ascending temperature or light intensity regimes, certain isolates were completely virulent (IT > 2) on cv. W-79/307 at either 26°C (Fig. 10.2) or 700 uEm-2s-1 (Fig. 10.4). This contrasted with the base population which was essentially avirulent (IT < 2) on this cultivar at these environments.

The IT was maximum for all the T- isolates when incubated at 20°C (Fig. 10.2). The IT of the 17 T- isolate was maximal (IT: 3) when incubated at lower temperatures (17° and 20°), decreased at 23° (IT: 1a) and was minimal (IT: 1) at incubation temperature of 26°C. The IT of the 26 T- isolate was maximum (IT: 4) at all temperatures of incubation while those of 20 T- and 23 T- isolates were reduced only when incubated at 26°C (IT: 2).

Similar trends were noticed in isolates selected at various light intensities (Expt. 10.2; Fig. 10.4). Both the 100 LI- and 300 LI- isolates had decreased IT (IT: 3 to either 1a or 1) when incubated in a light intensity above 300 uEm-2s-1, while the 700 LI- and 500 LI- isolates had maximal IT (IT: 4) at all four light intensities of incubation.

10.3.2 AGGRRESSIVENESS OF ISOLATES SELECTED AT SPECIFIC TEMPERATURE REGIMES (EXPT. 10.1) : The temperature isolates (T-), the incubating temperature and their interaction were significant (P < 0.001) sources of variation in all the four traits of aggressiveness (Table 10.2). While the temperature of incubation was the greatest contributor to variation in timing of the disease expression (IPF and LP1), isolates were more important sources of variation in the amount of disease produced (ULD and USM). When the variance due to the interaction component is added to the residual variance and the variance of the main effects re-calculated, these are still highly significant sources of variation (P < 0.001) in all the traits in both experiments. This permits discussion of the main effects independent of their interaction (Chandrashekar and Heather 1981).

Table 10.2 Analysis of variance for four traits of aggressiveness of four temperature adapted isolates of M. medusae on P. deltoides cv. W-79/307, incubated at four temperatures[ab] (Expt. 10.1).

Source	D F	IPF	LP1	ULD	USM
Isolate	3	26.87	106.64	124.88	45.82
Temperature	3	120.45	95.66	76.98	19.37
Isolate X Temperature	9	2.12	4.27	26.76	6.01
Residual†	304	0.17	0.85	0.83	0.43
Total†	319	1.61	2.96	3.48	1.71

a Using transformed values for ULD (sq. root) and USM (Log USM + 1).
b All the values are highly significant, P < 0.001.
† For USM, residual and total degrees of freedom is 176 and 191 respectively.

Table 10.3 Summary of analysis of variance for four traits of aggressiveness of four light intensity adapted isolates of M. medusae on P. deltoides cv. W-79/307, incubated at four light intensities[ab] (Expt. 10.2).

Source	D F	IPF	LP1	ULD	USM
Isolate	3	1.69*	39.24	72.64	45.38
Light intensity	3	0.29ns	57.37	27.90	25.07
Isolate X Light Int.	9	3.23	4.04	13.39	17.54
Residual†	224	0.45	0.62	0.84	0.08
Total†	239	0.56	2.14	2.57	2.01

a Using transformed values for ULD (sqaure root) and USM [Log (USM+1)]
b All unmarked values are highly significant, P < 0.001;
* significant, P< 0.025; ns - Not significant, P > 0.05.
† For USM, residual and total degrees of freedom is 176 and 191 respectively.

Figure 10.2. The level of the Infection Type (IT) and relative aggressiveness traits of temperature selected (T-) isolates (17, 20, 23 and 26) of M. medusae when incubated at four temperatures on P. deltoides cv. W-79/307. The four traits of aggressiveness were incubation period to flecking (IPF), latent period to production of first uredinium (LP1), uredinia produced per leaf disk (ULD), and urediniospores produced per sq. mm of leaf (USM). Square root transformed values for ULD and $Log_e + 1$ values for USM, are presented. Each value is a mean of 20 replications.

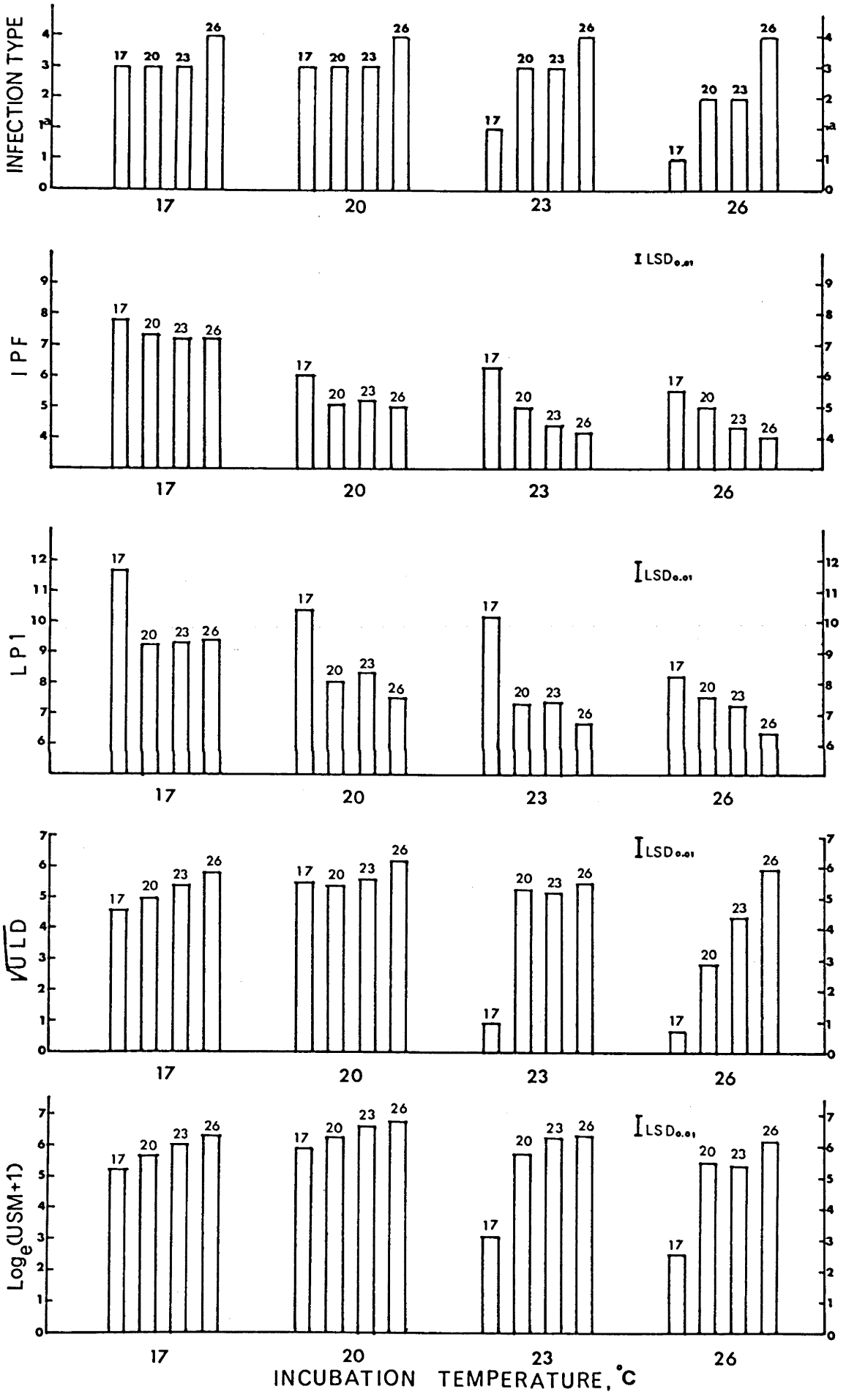

INFECTION TYPE
IPF
LP1
√ULD
Log$_e$(USM+1)
I LSD$_{0.01}$
17
20
23
26
INCUBATION TEMPERATURE, °C

Fig.10.2

Figure 10.3. Disease progress curves (basis number of uredinia per leaf disk) for the four temperature selected (T-) isolates (17, 20, 23 and 26) of M. medusae when incubated at four temperatures on P. deltoides cv. W-79/307.

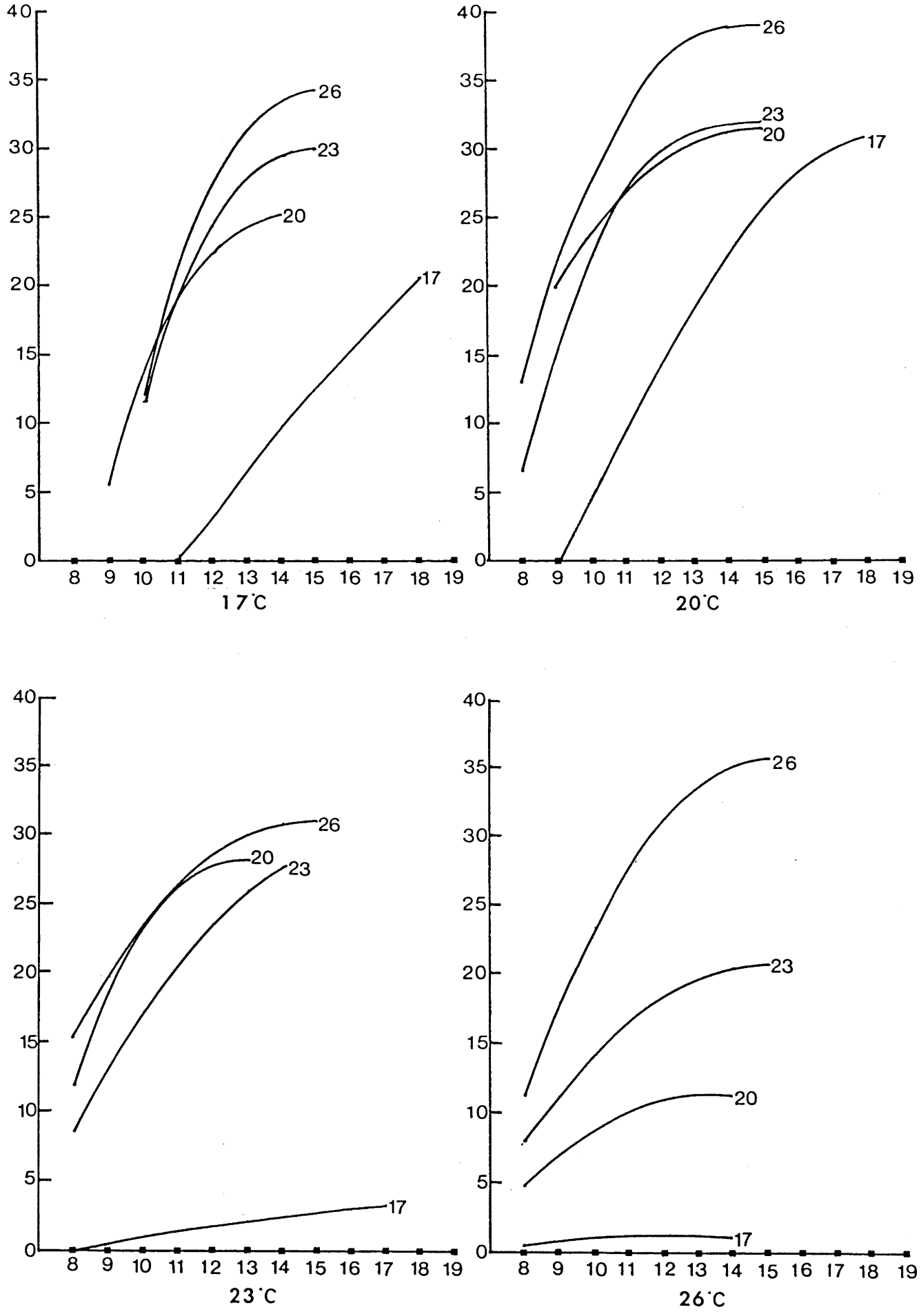


Fig.10.3 INCUBATION TEMPERATURE

At various temperatures of incubation there was a general consistency of ranking (26 > 23 > 20 > 17) for aggressiveness (basis ULD and USM) of the isolates (Fig. 10.2). However the 17 T- isolate was more aggressive (basis ULD and USM) when incubated at 17° and 20° C, than at 23° and almost avirulent at 26° C (Figs 10.2 and 10.3). The aggressiveness of the 20 T- isolate varied in a similar but less pronounced manner over the temperature scale, while the 23 T- and 26 T- isolates were highly aggressive at all the temperatures of incubation with the 26 T- isolate being the most aggressive (Figs 10.2 and 10.3). Such contrasts in behaviour of isolates may explain the significant interaction (temperatures of incubation x isolate) in the ANOVA despite the general constancy in ranking of the isolates.

Most T- isolates, but pronouncedly the 17 T- isolate, had a shorter IPF and LP1 when incubated at 26°C than at a lower temperature. In contrast, for most of the T- isolates (except 26 T- isolate), ULD and USM are significantly lower when incubated at 26°, rather than at a lower temperature (Fig. 10.2). At most temperatures of incubation the high temperature isolates (23 T- and 26 T-) had a shorter infectious period (i.e. time from uredinial eruption to disintegration) compared to low temperature isolates (Fig. 10.3). Irrespective of the temperature of incubation, the consistent ranking of the T- isolates for the rate of disease progress (26 > 23 > 20 > 17) (Fig. 10.3) parallaled that for ranking for ULD at the end of the monocycle suggesting that the latter is not an artefact of the time at which ULD was recorded. Although the curves for 26, 23 and 20 T- isolates were essentially quadratic, that for the 17 T- isolate was linear. This resulted from the low uredinial density produced by this isolate; this was associated also with a relatively long infectious period (Fig. 10.3).

AGGRESSIVENESS OF ISOLATES SELECTED AT SPECIFIC LIGHT INTENSITY REGIMES (EXPT. 10.2) : The light intensity (LI-) isolates, light intensity of incubation (except for IPF, $P > 0.05$) and their interaction were significant ($P < 0.001$) sources of variation for the traits of aggressiveness (Table 10.2). Light intensity of incubation was the major source of variation in the timing of disease expression (LP1), while LI- isolate was the most important contributor to variation in the

Figure 10.5. Disease progress curves (basis number of uredinia per leaf disk) for the four light intensity selected (LI-) isolates (100, 300, 500 and 700) of M. medusae when incubated at four light intensity regimes on P. deltoides cv. W-79/307.

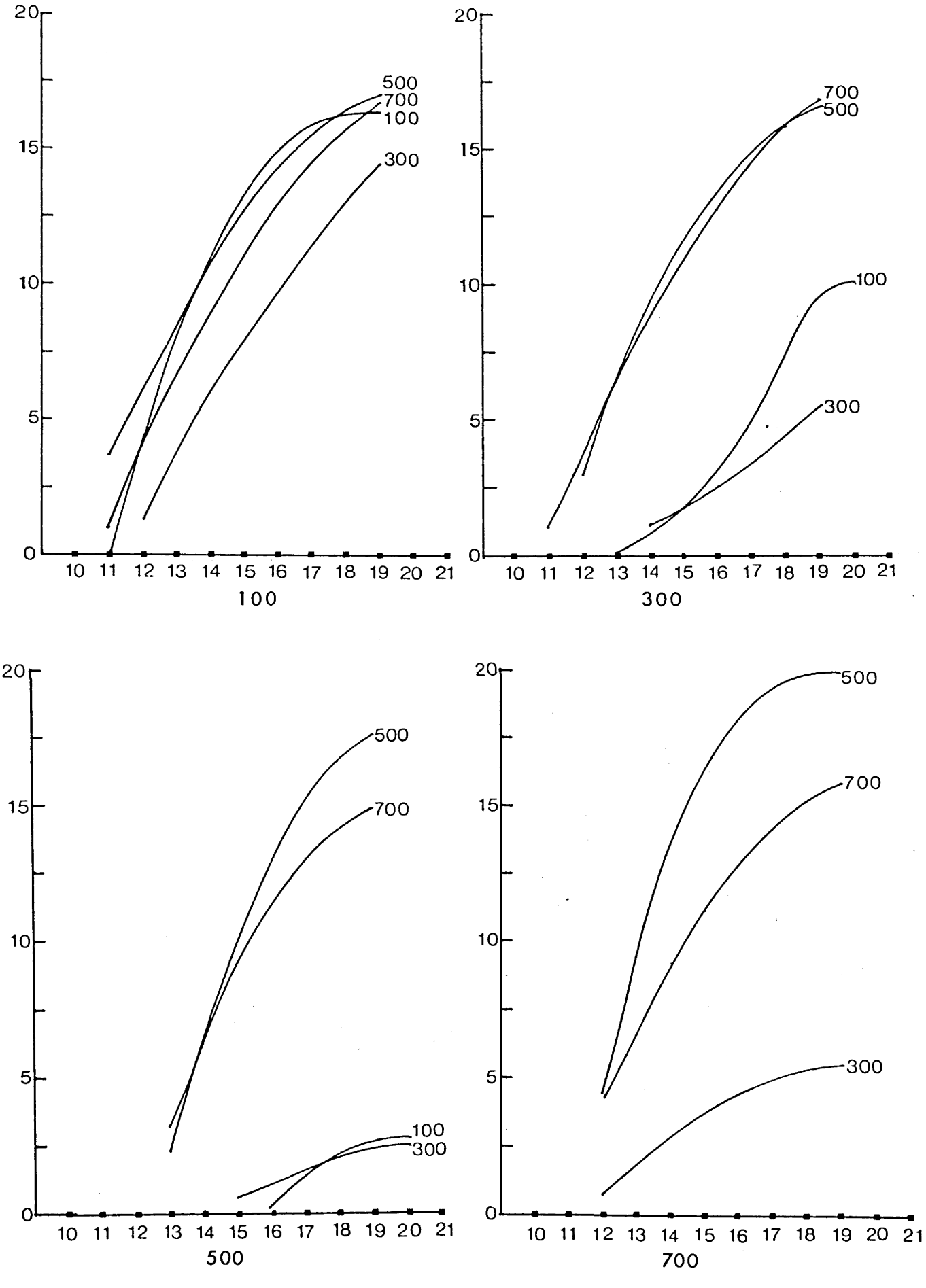


Fig.10.5 INCUBATION LIGHT INTENSITY

amount of disease produced (ULD and USM).

The 100 LI- isolate decreased in aggressiveness (ULD and USM) over the range (100-700 uEm^{-2}s^{-1}) of light intensity of incubation and was avirulent at 700 uEm^{-2}s^{-1} (Fig. 10.4 and 10.5). Although the 300 LI- isolate also decreased in aggressiveness (most traits) over the range of increasing light intensity of incubation, it was virulent at 700 uEm^{-2}s^{-1}. Again irrespective of the light intensity of incubation, the 500 and 700 LI- isolates were usually more aggressive (all the traits) than the 100 and 300 LI- isolates (except for IPF at 100 uEm^{-2}s^{-1}), with the 500 LI- isolate frequently more aggressive than 700 LI- isolate (Fig. 10.4). The ranking of the LI- isolates from the disease progress curves (Fig. 10.5), at each light intensity of incubation, in general parallels the ranking based on ULD of the isolates at the end of the monocyle (Fig. 10.4).

In a further study, when fresh leaf disks of cv. W-79/307 were inoculated with the urediniospores from the 26 T- isolate (of Expt. 10.1) and incubated at 16°C/700 uEm-2s-1, the isolate was avirulent. Similarly, the 700 LI- isolate was incompatible on this cultivar when incubated at 26°C/100 uEm-2s-1. This demonstrates the further specificity of the isolates towards a particular 'environment'.

In an additional experiment, urediniospores of the base population of race 4A were multiplied for 2 cycles on cv. I-488 at temperature/light intensity combinations of 26°C/100 uEm^{-2}s^{-1} or 16°C/700 uEm-2s-1 (environments at which this cultivar is compatible with race 4A). These urediniospores produced on cv. I-488 gave incompatible reaction when collected and deposited on disks of cv. W-79/307 which were incubated at 26°C/100 uEm^{-2}s^{-1} or 16°C/700 uEm^{-2}s^{-1}. This is suggestive of the role of the cultivar in mediating the environmental selection pressure on the pathogen.

10.4 DISCUSSION

The incubation of the leaf disks of all treatments in a uniform, congenial environment for 24 h, prior to allocation to specific regimes of temperature or light intensity, ensured that the virulence and aggressiveness recorded for the isolates in the various environments resulted from the post-penetration interaction of host, pathogen and environment (Singh and Heather 1982c).

While the base population and 17 T- and 100 LI- isolates were avirulent when incubated on cv. W-79/307 at 26°C and 700 $uEm^{-2}s^{-1}$ respectively, those isolates that were sequentially cultured at increasing temperatures or light intensities (26 T- and 500/700 LI-) were virulent at these environments (Figs 10.2 and 10.4). Thus these latter selected populations had 'adapted' to the higher temperature or light intensity of incubation. Thus, the population of race 4A of M. medusae, which is of mono-urediniospore in origin and reproduced asexually, responds rapidly by changes in its virulence to selection pressures of changing physical environmental conditions.

However, the nature of the isolates and the procedures employed (Fig. 10.1) to obtain them restricts the precise assessment of relative aggressiveness in certain results. For example each T- isolate is probably a population of mixed biotypes, each with a potential for virulence on cv. W-79/307 at one or more of the temperatures at which the isolates were selected. The proportion of biotypes is likely to vary between isolates depending on the selection pressure at each environment and such variation will affect the aggressiveness as measured by most traits. Further, the population of urediniospores selected as the 17 T-, 20 T-, 23 T-, 26 T- isolate had been cycled on cv. W-79/307 for 4, 5, 7 or 9 serial generations (Fig. 10.1). The aggressiveness (most traits) of certain races of M. medusae on a cultivar has been observed to increase with an increased number of serial generations on the cultivar (Chapter 7 and 8). This reservation would explain also the absence of dominance of 26 T- isolates in the base population despite its observed high aggressiveness at low temperatures.

Assessment of virulence on the qualitative infection type scale should be less affected by these reservations. Thus the virulence of the 26 T- (IT: 4) and the 700 LI- (IT: 3) isolates on cv. W-79/307 when incubated at 26°C and 700 uEm-2s-1 respectively, is beyond the potential of the biotypes constituting the base population from which these adapted isolates were obtained. Such differences in environmental specificities of the isolates would be due to genetic differences rather than physiological plasticity, because, subsequently biotypes with differing temperature optima were succesfully isolated in the base population (i.e. some biotypes were virulent at 16° but completely avirulent at 26°C) using the leaf replica technique (Appendix 3).

The reduction in aggressiveness, relative to that of the other isolates (basis ULD) of the 17 T- and 100 LI- isolates over the range of incubation temperatures 17-26°C and light intensities 100-700 uEm-2s-1, is evident throughout the course of the disease monocycle (Figs 10.2 and 10.4) and is not a consequence of the serial cycling of these isolates (each underwent three cycles only in their specific environments) nor is it an artefact of the time of assessment of ULD or USM. This reduction suggests that the particular isolates were selected to be most aggressive at their 'own' temperature or light intensity of incubation, i.e. the level of the environmental factor at which they were cycled on the cultivar. In a conceptual sense this agrees with the reports that some biotypes of plant pathogens were most aggressive on those culitvars on which they were isolated (Caten, 1974; Clifford and Clothier, 1974; Wolfe and Barrett, 1980).

The aggressiveness of the 26 T- or the 500/700 LI- isolate was independent of the respective temperature or light intensity regimes over which they were incubated; further under these regimes they are the most aggressive among their particular group (temperature or light intensity) of isolates. However the biotypes which make up these isolates do not constitute the bulk of the population of the 17 T- isolate or 100 LI- isolates, otherwise these latter isolates would be highly aggressive and also highly virulent (IT > 3) at the high temperature or light intensity of incubation. Possibly, the high uniform aggressiveness of the high temperature and high light intensity

isolates under certain condition of incubation results in part from the adaptation to cv. W-79/307 following their cycling on this culitvar for nine generations. However both the 26 T- and 700 LI- isolates were avirulent on cv. W-79/307 when incubated at 16°C/700 uEm-^{2}s-^{1} and 26°C/100 uEm-^{2}s-^{1} (temperature/light intensity) respectively or when adapted on cv. I-488 at these environments. These observations indicate that adaptation for virulence, and probably for aggressiveness, involves a cultivar/environmental variable/isolate interaction.

The observations in this system could explain also the specificity of the isolates of certain other plant pathogens towards their 'own environment' (Shepherd et al, 1973; Hill and Nelson, 1976; McCoy and Blakeman, 1976). Line and Bugbee (1964) observed that isolates of Puccinia graminis f.sp. tritici that were adapted in their germination to cold temperature (3-5°C) after selection at this temperature for more than 20 cycles, lost their ability to germinate at normal temperatures (20-25°C). This contrasts with the results of present study, where the isolates adapted for virulence at high temperature or light intensity, were virulent at low temperature and low light intensity.

The present results suggest that populations of leaf rust biotypes, with the potential to show comparable differences in virulence and aggressiveness, could be obtained by a combination of cultivar and environmental selection pressures acting on a base population and/or on mutants which arise during multiplication of the population. Due to the genetic heterogeneity of cultivars in natural populations of poplar, and the diurnal, seasonal and spatial variation in physical environmental conditions in nature, it is likely that over extensive areas of the host and/or for long periods, the selection pressure will be disruptive rather than directional (cf. Mather, 1955; Thoday, 1972). As this rust responds rapidly to such selection, the selection pressure of the variable environment is likely to result in polymorphism in the pathogen population (Haldane and Jayakar, 1953) as no single phenotype would be the most fit to survive throughout the season or area. Such balanced and transient polymorphism of the pathogen populations are essential requirements in the stability of the pathosystem (Leonard and Czochor, 1980).

10.5 SUMMARY

Race 4A of M. medusae produces an incompatible reaction on P. deltoides cv. W-79/307 when incubated at high temperature (26°C/100 uEm-2s-1) or high light intensity (17°C/700 uEm-2s-1), but a compatible one at low temperature and light intensity (17°C/100 uEm-2s-1). When, in separate studies, a population of this race was sequentially cultured on detached leaves, at increasing temperatures (17, 20, 23, 26°C) or light intensities (100, 300, 500, 700 uEm-2s-1), isolates that were adapted to each of these regimes were selected. Such isolates, particularly those from low temperature and light intensity, exhibited some specificity to their 'own' environments, although isolates selected at 26°C and 500 uEm-2s-1 were most aggressive at all temperature and light intensity regimes respectively. Such adaptation was observed to result from pathogen response to host-mediated environmental selection pressure. The poplar leaf rust readily adapts to varying environmental conditions and it is suggested that physical environmental variables may be important selective forces in the regulation of the pathosystem, as spatial and temporal heterogeneity of the environment in nature may result in polymorphism of the pathogen by disruptive selection.

CHAPTER 11

TEMPERATURE - ACTIVATION OF INCOMPATIBILITY AND INDUCED RESISTANCE TO *MELAMPSORA MEDUSAE* IN POPLAR

11.1 INTRODUCTION

In the pathogen, temperature sensitive biotypes are important in studies on basic aspects of pathogenesis. Similarly, the temperature-sensitive resistance in the host is useful in understanding the incompatibility phenomena (section 10.3) (Seevers and Daly, 1970; Ellingboe, 1977; Jones and Deverall, 1977; Vanderplank, 1978). Studies on inducing resistance to a virulent race by prior inoculation of a host with an avirulent race facilitate further analysis of resistance mechanisms and have epidemiological and evolutionary implications (Johnson & Allen, 1975; Harlan, 1976; Kuc, 1983; Ouchi, 1983).

The aim of this chapter was to study the chronology of activation of temperature sensitive resistance in a clone of poplar to *M. medusae* and to test the occurrence of induced resistance with temperature-activated incompatibility.

Race 4C of *M. medusae* is virulent (+) on *P. deltoides* cv. W-79/307 at 16° but avirulent (-) at 26°C (Plate 11.1), while race 4M (actually race 4M2 ; Chapter 5 and 6) was found to be virulent (+) on this cultivar at both temperatures (Table 11.1).

Table 11.1 Virulence (+) or avirulence (-) of the two races of *M. medusae* on cv. W-79/307 at 16° and 26°C.

Pathogen / Temperature	Race 4C	Race 4M
16°C	+	+
26°C	-	+

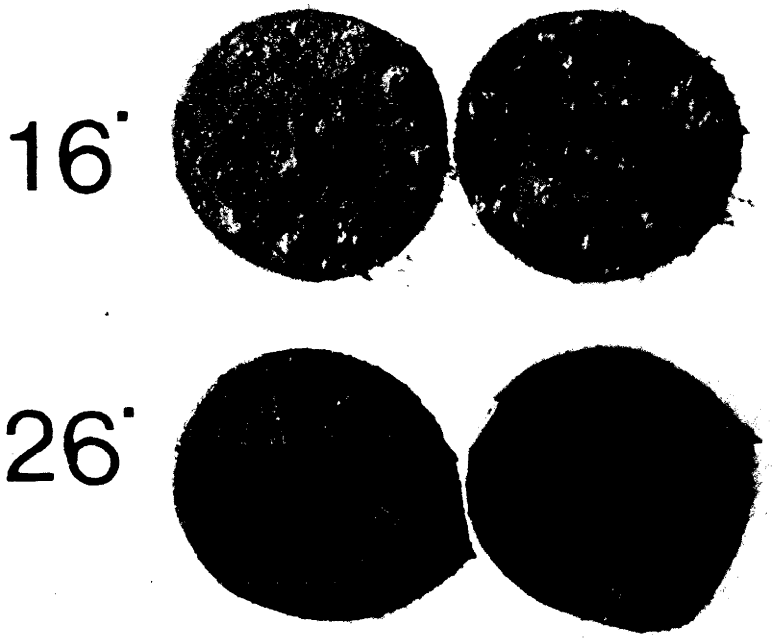


Plate 11.1 The temperature-sensitive reaction of race 4C of M. medusae on leaf disks of P. deltoides cv. W-79/307, when incubated at 16° or 26°C.

The timing of the temperature activation of resistance or susceptibility to race 4C in cv. W-79/307 (Expt. 11.1), and the induction, by prior inoculation with race 4C and incubation at 26°C, of resistance *in vitro* of this cultivar to race 4M at 16° and 26°C were investigated (Expt. 11.2).

11.2 MATERIALS AND METHODS

11.2.1 THE PATHOGEN ISOLATES : Races 4C and 4M, mono-uredinium derived biotypes of race 4A of *M. medusae*, were isolated by the 'leaf replica technque', designed to separate avirulent, virulent and temperature sensitive biotypes within a fungal population (Appendix 3). While race 4C was isolated for the temperature sensitivity of its virulence on cv. W-79/307, race 4M was isolated as a spontaneous mutant, virulent towards *P. deltoides*, from within race 4A (Chapter 4 and Appendix 3). Subsequently, race 4M was found compatible with cv. W-79/307 at both 16° and 26°C.

The races were multiplied separately on detached leaves of *P. x euramericana* cv. I-488 (section 2.4.1). The freshly harvested urediniospores were dried over silica gel (24 h) and P_{2O5} (24 h).

11.2.2 THE INOCULATION PROCEDURE: Leaf disks (1.70 cm^2), punched from surface sterilised leaves of uniform age (*c.* 3 months), of clonal plants of cv. W-79/307, raised in rust-free glass house (section 2.2), were bulked and shaken in a polythene bag to ensure randomisation. One hundred and fifty leaf disks were initially inoculated (abaxial face) with urediniospores (4 mg) of race 4C in a spore settling tower and five replicate leaf disks were randomly placed on plastic foam soaked with gibberellic acid solution in separate Petri dishes. Certain Petri dishes, each containing leaf disks which had not been inoculated, were kept at 16° or 26°C ($_{+1°C}$), to be inoculated subsequently with race 4M. All the leaf disks were incubated initially for 4 h at 16°C under diffused light (30-50 $uEm^{-2}s^{-1}$) to allow satisfactory germination (> 95%) and penetration of the inoculated disks by the rust (Omar, 1978; Singh and Heather, 1982c). Later, the Petri dishes were transferred to

Figure 11.1 Schematic diagram of the experimental procedure to test the timing of compatibility or incompatibiity in P. deltoides cv. W-79/307 to race 4C.

Race 4C was inoculated on leaf disks of cv. W-79/307 and half the number of disks (in Petri dishes) were incubated at 16° and other half were incubated at 26°C. Each day over a twelve day period a Petri dish with 5 disks from each temperature regime transferred to the other temperature (16° to 26° and 26° to 16°C) from one to twelve days of inoculation, while the control plates with 10 disks each were not transferred.

Figure 11.2. Procedure followed to test the occurrence of induced resistance to race 4M of M. medusae by temeperature activated incompatbility between race 4C and cv. W-79/307.

Race 4C was initially inoculated on disks of cv. W-79/307 and half the number of disks were incubated at 16° or 26°C. Similarly, uninoculated leaf disks were also incubated at these temeperatures. After four days of incubation, race 4M was inoculated on all disks and half number of disks from each treatment were incubated at either 16° or 26°C.

Fig 11.1

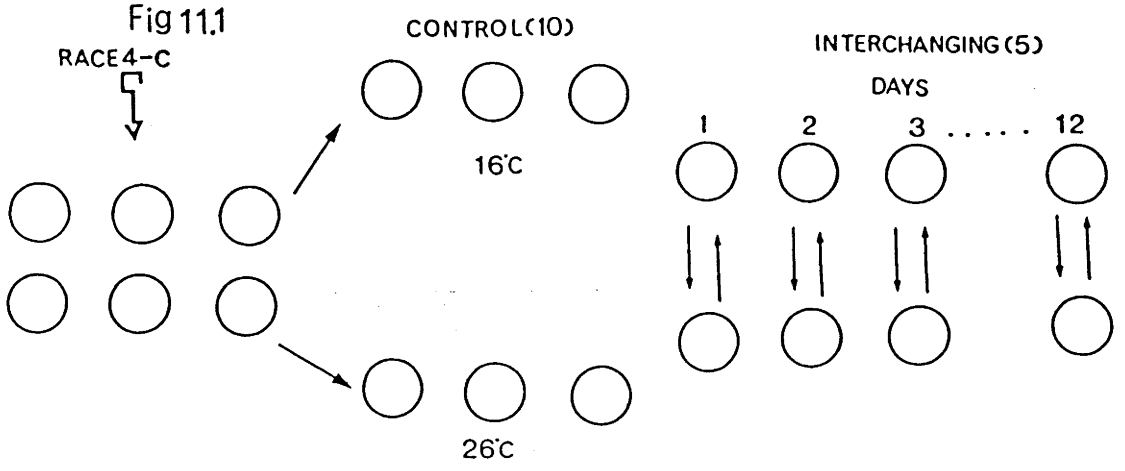
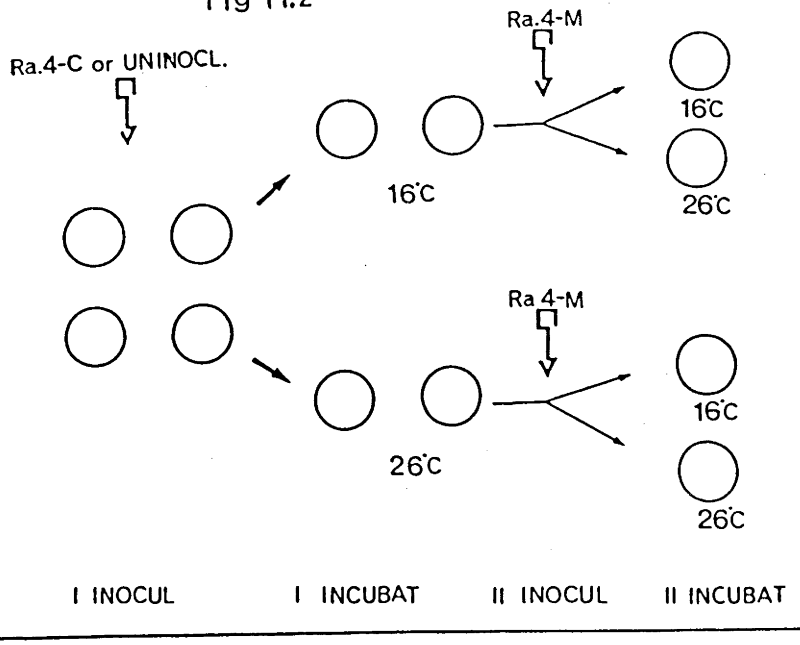


Fig 11.2



separate growth cabinets, maintained either at 16 or 26°C, light intensity of 100 uEm-2_s-1 and photoperiod of 16 h.

11.2.3 TRANSFER OF LEAF DISKS ACROSS TEMPERATURES (EXPT.11.1) : Single Petri dishes (each with 5 leaf disks) from two groups of 16° or 26°C, were transferred in a reciprocal fashion daily between the two temperature treatments, over a period of 12 days (day of inoculation-day 0), (Fig. 11.1). Some Petri dishes were maintained at 16° and 26°C throughout, without interchanging, to serve as controls. The timing of activation of in/compatability was assessed on these leaf disks which were moved from one temperature to the other.

11.2.4 CROSS INOCULATION (EXPT.11.2): The urediniospores (4 mg) of the compatible race 4M were deposited on certain leaf disks either previously inoculated (race 4C) or uninoculated, which had been incubated of four days either at 16° or 26°C (Fig. 11.2). Subsequently, following incubation (4 h, 16°C, diffused light; to allow the urediniospores of the newly deposited race to germinate and penetrate), disks were randomly allocated to separate Petri dishes (5 disks per treatment), and incubated at either 16° or 26°C (Fig. 11.2) (Expt. 11.2). This study enabled testing of whether the resistance, induced by race 4C on this cultivar at 26°C, was effective also against at race 4M, which was compatible with this cultivar at higher temperatures.

11.2.5 OBSERVATIONS ON DISEASE: The Infection Type (IT) on a 0-4 scale, of increasing disease severity (section 2.6.1) was recorded, after 18 days of incubation for Expt. 11.1, and 22 days of incubation (18 days after second inoculation) for Expt. 11.2. An IT of < 2 was classed as an incompatible reaction.

11.3 RESULTS

11.3.1 TIMING OF INCOMPATIBILITY OR COMPATIBILITY (EXPT.11.1) : On the control leaf disks of W-79/307, maintained at a constant temperature, race 4C was compatible (IT-3), or incompatible (IT-0), when incubated for 18 days at 16° or 26°C respectively (Table 11.2).

Table 11.2 Disease reaction (IT) [1] of P. deltoides cv. W-79/307 inoculated with M. medusae race 4C, incubated at 16° (compatible temperature) or 26°C (incompatible temperature) and subsequently reciprocally transferred between the temperature regimes over 12 days.

			Days at I temperature profile											
Control	IT	Transfers	1	2	3	4	5	6	7	8	9	10	11	12
16°	3	16 -> 26	1^a	1^a	1^a	1-2	2	2	3	3	3^b	3^b	3^b	3^b
26°	0	26 -> 16	1	1	1	1	0-1	0-1	0-1	0	0	0	0	0

1 Infection Type on a increasing scale of 0-4, asessed 16 days after inoculation
IT-0 : immune, with no macroscopic symptoms, 1 : resistant, chlorotic/necrotic spots, 1^a : 1 or 2 small uredinia, 2-4 : increasing number uredinia. 'b' indicates, relatively large uredinia.

Race 4C gave an incompatible reaction (IT-1[a]) when incubated on disks of cv. W-79/307 at 16°C for four days or less, prior to transfer to 26°C but a compatible reaction (IT > 2) when comparable disks were incubated at 16°C for five or more days, prior to transfer to the higher temperature (Table 11.2). The infection type in the latter disks increased (IT-2 to 3+) with an increased period of incubation at the lower temperature prior to transfer to 26°C, while the control disks incubated at 16°C throughout, had an IT-3 (Table 11.2).

In contrast race 4C demonstrated incompatibility (IT < 1) on the leaf disks of cv. W-79/307, which were initially incubated at 26° and subsequently transferred to 16°C (Table 11.2). The degree of incompatibility increased (IT 1 to 0) with increasing periods of incubation at the higher, prior to tranfer to the lower, temperature (Table 11.2). These results were reproduced when the study was repeated. Also, in a separate study, when similar reciprocal transfers were made at hourly intervals, at least 15 h of incubation at 26°C was necessary for the activation of incompatibility when the cultivar was transferred subsequently to the low temperature.

The second experiment examined the effectiveness of such temperature induced resistance by race 4C against race 4M, which is compatible on cv. W-79/307 at both temperatures.

11.3.2 TEMPERATURE ACTIVATED INDUCED RESISTANCE : When incubated continuously at 16°C, inoculated leaf disks of cv. W-79/307 gave an IT-4 with race 4M, an IT-3 with race 4C, while with the former race the IT was higher under continuous incubation of 16° (IT-4) rather than 26°C (IT-2) (Tables 11.2 and 11.3). Incubation of uninoculated disks at 26°C for four days, prior to inoculation with race 4M and subsequent incubation at 16°C, reduces the level of disease intensity (IT-2/3) when compared with that in disks maintained at 16°C (IT-4) throughout the pre- and post-inoculation periods (Table 11.3).

The subsequent inoculation with race 4M of disks, previously inoculated with race 4C and incubated for 4 days at 26°C, produced an incompatible reaction with IT-1 or IT-0 depending on whether following this second inoculation the disks were incubated at 16° or 26°C (Table 11.3). However when the leaf disks were inoculated with race 4C and

Table 11.3 Disease reaction (IT; 0-4 scale of increasing disease severity) of *P. deltoides* Cv. W-79/307 to race 4M of *M. medusae*, with or without prior inoculation by race 4C and incubation at pre- and post-second inoculation temperatures of 16° or 26°C.

	Pre-second inoculation temperature			
	No prior inoculation		Prior inoculated (race 4C)	
Post-second inoculation tempr.	16°	26°	16°	26°
16°	4	2-3	4	1
26°	3	2	4	0

Note: When inoculated independently, race 4C is compatible on this cultivar at 16° but incompatible at 26°C, while race 4M is compatible at both temperatures of incubation (Table 11.1).

incubated at 16°C for four days, prior to inoculation with race 4M, the leaf disks produced an IT - 4 irrespective of the temperature of incubation following the second inoculation (Table 11.3).

11.4 DISCUSSION

Incubation at 26°C for 15 h of leaf disks of cv. W-79/307 inoculated with race 4C is sufficient to induce incompatibility to this race. Further, this rapidly induced incompatibility is irreversible i.e. the transfer after 24 h of inoculated disks from 26° to 16°C does not result in compatibility.

Leaf disks of cv. W-79/307, in which temperature sensitive incompatibility has been induced by inoculation with race 4C and incubation at 26°C for four days, demonstrate either high resistance (IT-0) or hypersensitive (IT-1) reaction when subsequently inoculated with race 4M and incubated at 26° or 16° respectively i.e. temperatures at which this cultivar is normally compatible with this race (Table 11.3). Hence this temperature (26°C) induced incompatibility is irreversible not only for the original race (4C) but it is epistatic to the potential compatibility of the cultivar with race 4M at both high (26°) and low (16°C) temperatures (Kuc, 1982; Ouchi, 1983). Further, this epistatic incompatibility, induced by race 4C, was partly systemic when half leaf or discrete zone inoculations are made on whole leaves (details not presented here).

In contrast to these characteristics of the incompatible reaction, the compatiblity of race 4C with cv. W-79/307 developed only in the disks which had been incubated for at least four days at 16° (the compatible temperature) prior to transfer to 26°C (the incompatible temperature for this cultivar/race complex). Further the degree of compatibility in the complex increases (IT 2 to 3+) with the increased period (up to 9 days) of incubation of the inoculated disks at 16°C prior to the transfer to the higher temperature. This observation resembles the effect on infection type of time of transfer from a low to a high temperature regime of plants of the wheat variety Rego inoculated

with Puccinia striiformis West. (Brown & Sharp, 1969). In a similar study with cv. McMurachy of wheat and P. graminis f. sp. tritici, t' transfer of seedlings from an incompatible (16°) to a compatible (22°C) temperature a day prior to development of necrotic flecking, resulted in a susceptible reaction but when transferred at the time of flecking or later, a resistant resistant reaction always resulted. An alternation of 22° (16 h) and 16°C (8 h) also resulted in complete resistance (Forsyth, 1956).

Similarly with P. striiformis on certain varieties of wheat, short exposures to contrasting temperatures at critical stages in the infection process resulted in significant changes in infection type, although the relative significance of the pre- and post-inoculation temperature regime in determining the infection type, depended on the variety of wheat used (Brown & Sharp, 1969). By heat treatment, Tani et al (1975) determined that the cellular conditioning in oat leaves towards the resistant response was completed between 8-12 h after inoculation with an incompatible race of Puccinia coronata Corda f. sp. avenae Avls. However, in contrast to the present results, the resistance to race 56 of P. graminis f. sp. tritici in wheat varieties containing the Sr6 gene*, when incubated at an incompatible temperature (20°C) for four days, changes to susceptibility following transfer of such plants to a compatible temperature (26°C) (Seevers & Daly, 1970). Such contrasts suggest that the mechanisms of incompatibility operating in these systems may differ.

Race 4C induced higher levels of disease on cv. W-79/307 when inoculated leaf disks are incubated at 16° for 9-12 days and then transferred to 26°C (IT-3b) than when such disks are maintained at 16°C throughout (IT-3). Thus the higher temperature, which favours avirulence in this cultivar/race combination, also favours aggressiveness once the avirulence in the combination has been overcome.

The observed patterns of reactions in this system suggest that immediate recognition is for incompatibility - a rapid irreversible, epistatic and possibly a single step process, rather than compatibility- a slower, reversible (at less than four days) and possibly a multi-step process. This conclusion accords with the proposal of Ellingboe (1977) that in host pathogen systems, recognition is for incompatibility which

*(which confers incomplete resistance)

results from a major gene system superimposed on a basic compatibility system. Parlevliet (1983a) has discussed the relevance of such a system in host-pathogen coevolution. Similarly the present results would conform to the 'stop signal' concept advanced by Person & Mayo (1974) to explain the epistasis of incompatibility.

Hypersensitive necrosis develops after five to six days in disks of cv. W-79/307 inoculated with race 4C and maintained at 26°C for 24 h prior to transfer to 16°C. However the resistance (IT 0/1) of such disks to this race has been decided in less than 24 h (Table 11.2) and to race 4M in less than 4 days. (Table 11.3). Such resistance cannot be caused by the macroscopically expressed hypersensitivity observed five days later. This agreess with the controversial suggestion of several authors that hypersensitivity is the result rather than the cause of disease resistance (Brown et al, 1966; Kiraly et al, 1972).

11.5 SUMMARY

When, over a 12 day period, groups of leaf disks of P. deltoides cv. W-79/307 inoculated with race 4C of M. medusae, which gives a compatible reaction when incubated at 16°C (Low Temperature, LT) but an incompatible reaction at 26°C (High Temperature, HT), were reciprocally transferred daily between the temperature regimes, incubation for as short as 15 h at HT resulted in incompatibility which was not reversed by subsequent incubation at LT. In contrast, incubation of the inoculated disks at LT for at least four days was necessary for the development of a compatible reaction following transfer to HT. Further, the incompatibility induced in disks by inoculation with race 4C and incubation at HT is epistatic to the expected compatibility following subsequent inoculation with race 4M, a temperature non-sensitive biotype. The rapidity, irreversibility and epistatic nature of the temperature induced incompatibility suggests that, in this pathosystem, recognition is for incompatibility. The temperature sensitivity of the system and the occurrence of induced resistance has potential epidemiological importance.

SECTION III

SECTION III

CHAPTER 12

GENETICS OF QUALITATIVE AND QUANTITATIVE FORMS OF RESISTANCE TO *MELAMPSORA MEDUSAE* IN *POPULUS DELTOIDES*

AN INTRODUCTION

12.1 INTRODUCTION : Historically, agriculturists and foresters have recognised differences in disease severity among plants and the earliest records of such observations date back to the time of Theophrastus (372-287 B.C.) (Day, 1974). The breeding for disease resistance based on genetic priniciples, however, did not begin until early this century when Biffen (1905) demonstrated that resistance to *Puccinia glumarum* (*P. striiformis*) in wheat followed Mendel's laws of inheritance and was determined by a single recessive gene. Thus, it was realised that in crop plants, artificial hybridization and selection could be employed to breed for resistance to diseases. Breeding and selection for resistance to many diseases has been practised for some time in most field crops and in a few trees. While some, or most, of the resistant cultivars have been successful in keeping the disease levels below economic threshold limits (Russel, 1978; Johnson, 1983), there are many instances where such cultivars have subsequently succumbed to the disease with considerable economic damage (Marshall, 1977).

An understanding of inheritance and genetic aspects of resistance in the host is an essential requirement for planning breeding and deployment strategies. The genetics of resistance to pathogens has been increasingly studied in numerous plants and some excellent reviews and discussions are available (e.g. Vanderplank, 1968; Hooker and Saxena, 1971; Person and Sidhu, 1971; Russel, 1978; Nelson, 1979; Wilcoxson, 1981; Parlevliet, 1981, McIntosh and Watson, 1982) and thus this is not discussed in detail here.

With rust, resistance in the host can be qualitative, where the reaction is complete, usually marked by hypersensitive or low infection type, or can be quantitative, where resistance is partial or incomplete, characterised by longer latent period, fewer uredinia and lower sporulation.

12.2 INHERITANCE OF QUALITATIVE RESISTANCE :

12.2.1 NATURE OF GENES CONTROLLING QUALITATIVE RESISTANCE : The genetic information on resistance relating to dominance or recessiveness, presence of additive and epistatic (non-allelic) interaction is helpful in tailoring breeding programs. For example, dominance or recessiveness of resistance indicates the proportion of the segregating population that is likely to be resistant. Also if resistance is recessive, selection for resistance in autogamous crops cannot be exercised until the F2 generation. Even if the resistance is dominant, the selected plant from the segregating population may not necessarily be homozygous for such resistance alleles. The presence of additive genes indicates that resistance levels could possibly be increased by further hybridization and would indicate the basis for predicting advances under selection (Mather, 1979).

Most studies on qualitative resistance in agricultural crops have suggested that such resistance is controlled by dominant genes (Person and Sidhu, 1971; Day, 1974). However there are also many instances where such resistance is controlled by recessive genes (Hooker and Saxena, 1971; Day, 1974; Russel, 1978; McIntosh and Watson, 1982). Qualitative resistance has also been generally observed to be controlled by multiple alleles in a few well studied crop systems such as flax and maize (Flor, 1971; Hooker and Saxena, 1971; Day, 1974), and thus it may not be possible to combine all such genes into one cultivar genotype.

12.2.2 NUMBER OF GENES CONTROLLING QUALITATIVE RESISTANCE : The qualitative resistance is usually controlled by oligogenes (few genes with major effect). The information on number of genes (oligo or poly) is useful in determining the size of segregating and selection

population to be retained during breeding. A single dominant gene is more common than other forms of genetic control of qualitative resistance in field crops (Day, 1974).

Person and Sidhu (1971), in a comprehensive review of inheritance studies to that date, suggested that in many instances, failure to demonstrate the control of resistance by a single dominant gene, resulted from employing bulked or field inoculum rather than pure races, for testing and thus ratios resulting from epistasis would be observed. Nevertheless, there are many instances of resistance in plants to diseases being controlled by recessive, modifier, inhibitor or cytoplasmic genes (Hooker and Saxena, 1971).

12.2.3 STABILITY OF QUALITATIVE RESISTANCE : The qualitative resistance is relatively easy to identify and handle in breeding programs. The race specificity of such resistance is also qualitative and thus is more pronounced than those in quantitative resistance (Parlevliet and Zadoks, 1977). Frequently, such qualitative resistance controlled by oligogenes are easily overcome by a corresponding mutation for virulence and thus may be short lived (Nelson, 1978; Parlevliet, 1981); however there are many instances where such qualitative resistance has proved to be stable (Watson, 1970; Johnson, 1983).

12.2.4 INHERITANCE OF RESISTANCE TO DISEASES IN FOREST CROPS : In trees, the literature on inheritance of resistance to diseases is scant, while there are few reviews on disease resistance studies on trees (Bingham *et al*, 1972; Heybroek *et al*, 1982). Muhlelarsen (1963) from a field analysis of F1 progeny of crosses *P. deltoides* X *P. nigra* and *P. nigra* X *P. trichocarpa* exposed to a natural populations of unidentified species of *Melampsora* concluded that resistance was dominant, controlled by one or more genes. In sugar pine (*Pinus lambertina* Dougl.), the hypersensitive reaction in needle tissue to fusiform rust is apparently conditioned by a single dominant gene (Kinloch, 1982). Resistance in *Thuja* spp. to *Keithia thujina* Durand was also controlled by a single dominant gene (Soegaard, 1966). Heimburger (1962) hypothesised that an infected needle-shedding type resistance to *Cronartium ribicola* J. C. Fisch. ex Rabenh. was determined by a simple recessive gene in *Pinus*

wallichiana Jacks.

12.3 INHERITANCE OF QUANTITATIVE RESISTANCE : While qualitative resistance has a clearcut reaction of incompatibility, quantitative resistance is a compatible reaction characterised by degrees of susceptibility measured in terms of many traits including latent period, uredinial number, sporulation, rate of disease progression, area under disease progress curves etc. (Johnson and Taylor, 1976; Rees et al, 1979; Parlevliet, 1979; Wilcoxson, 1981). Quantitative resistance, like yield, is often continuously distributed among the segregating progeny and thus is usually polygenically inherited (genes with minor effects) (Kulkarni and Chopra, 1980; Wilcoxson, 1981). While genetic studies on qualitative traits follow simple Mendelian analysis, that of quantitative traits require biometrical approach.

The shift from the use of qualitative to quantitative forms of resistance to control disease stems from few past failures of qualitative resistance due to new virulent races, coupled with the genetic uniformity of the crops (Marshall, 1977). Thus quantitative resistance, which slows down the epidemic by lower rate of infection rate, longer latent period and lower sporulation (Parlelvliet, 1979; Wilcoxson, 1981), was conceived to be better suited to 'manage' the pathogen by keeping its activity to moderate levels. Further, since such resistance does not threaten the survival of pathogen, the latter may not develop 'super-races'. Also, such resistance, when controlled by many genes, is thought to be more stable as it requires many steps of change in the pathogen to overcome the resistance (Parlevliet, 1983a). Many authors, however, have suggested that such quantitative resistance need not necessarily be stable (e.g. Nelson, 1978; Vanderplank, 1978; Johnson, 1983).

Nevertheless, quantitative resistance has conferred apparent stability to many pathosystems including potato-Phytopthora infestans, maize-P. sorghi, and barley-P. hordei (Parlevliet, 1981). Breeding and selection for quantitative forms of resistance in wheat against P. graminis tritici (Skovmand et al, 1978; Rees et al, 1979), oats against P. coronata (Simons, 1975), barley against P. hordei (Parlevliet, 1977;

Johnson and Wilcoxson, 1979) and in coffee against Hemileia vastatrix Berk. et Br. (Eskes, 1983b), is being pursued. The use of quantitative forms of resistance is suggested also in forest tree systems (Heybroek et al, 1982; Thielges, 1984).

One of the merits claimed in the use of quantitative resistance is that it is race non-specific viz., there is no differential race x cultivar interaction, and ranking of cultivars does not change with races (Vanderplank, 1968). Considerable evidence opposing this view has accumulated, wherein cultivars exhibiting quantitative specificity with races for traits like latent period, sporulation etc. are known (Section I) (Clifford and Clothier, 1974; Johnson and Taylor, 1976; Parlevliet, 1979; Chandrashekar, 1981; Singh and Heather, 1983). Also, there is some evidence that quantitative resistance may erode over time due to selection pressure on the pathogen resulting in increased aggressiveness (Caten, 1974; Clifford and Clothier, 1974; Wolfe and Barrett, 1980; Chapters 7 and 8 of this thesis).

12.3 STRATEGIES IN USE OF HOST RESISTANCE : There are various ways in which resistance, either qualitative or quantitative, has been used to control plant diseases. Again several comprehensive reviews are available (e.g. Vanderplank, 1968; Watson, 1970; Day, 1974 and 1977; Robinson, 1976; Russel, 1978; Parlevliet, 1981; McIntosh and Watson, 1982) and hence are not detailed here.

Traditionally, since early this century, efforts have been directed to breed plants with single gene resistance and these are usually deployed as pure line cultivars in autogamous crops. As explained elsewhere (Appendix 7), due to the recurring exercise of such breeding ('boom and bust' cycle), many alternatives have aimed at relatively 'durable' or stable forms of resistance in crop plants (Johnson, 1983) and in trees (Heybroek et al, 1982) including those in poplar to leaf rusts (Thielges, 1984).

Parlevliet (1981) and Thielges (1984) have discussed in detail many of the strategies of employing host resistance to control diseases. Parlevliet (1984) regards the most common strategy as no

strategy at all, where at one time a few qualitative resistance genes are used over large areas and are quickly replaced when such resistance is 'broken down'. Another approach is to precisely monitor the virulence composition of the pathogen population in relation to the resistance gene being used and breeding is geared towards keeping a step ahead of the pathogen.

The third strategy is to 'pyramid' many qualitative resistance genes into a single cultivar. The resistance of such a cultivar is thought to be potentially stable as it requires concurrent multiple changes in the genotype of the pathogen to overcome all resistance genes. Even if such super-races do appear, due to the assumed residual effect of 'defeated' resistance genes, the cultivar would not be highly susceptible (Nelson, 1979). Person et al (1976) have questioned the validity of such an approach which they claim is based mainly on genetic probabilities.

The strategy of using polygenic resistance has been discussed earlier in relation to quantitative forms of resistance (section 12.2). Due to the complexities inherent in quantitative traits, such an approach requires precise and elaborate conditions of testing and observation, and to keep the environmental variance at a minimum during testing.

The approach of using mosaic patterns of cultivars in time and space, has gained prominence recently. This aims at providing a diverse host environment by deploying number of cultivars each carrying different resistance gene simultaneously in various areas or in succession in an area, or both (Parlvliet, 1981). Obviously, in tree systems, mosaic patterns over time are not a feasible proposition. Multilines and cultivar mixtures are other alternatives and considerable discussion on their merits and problems in controlling diseases have been made (Vanderplank, 1968, Browning and Frey, 1969; Parlevliet, 1981; Wolfe, 1984). Marshall and Pryor (1978) concluded that success of multiline depends on strength of cost of virulence and number of component lines in the mixture.

Cultivar mixtures, where the background genotypes also differ

in addition to qualitative resistance genes, has been proposed to manage disease in a few systems (Wolfe and Barrett, 1980). The merit of cultivar mixtures over multilines, may be that, due to heterogeneity of such background genotype in the host, the aggressiveness in addition to virulence of the pathogen may be kept to moderate levels (Leonard, 1969).

Monitoring the pathogen population for its virulence, aggressiveness, to understand strategies of its evolution and to predict potential population shifts, are important requirements for success of most strategies (Parlevliet, 1981; Kranz, 1983; Wolfe et al, 1983). In forest tree systems, specific considerations of the complexities in this system, along with economic, logistic and management factors should be borne in mind for developing any strategy (Libby et al, 1969; Thielges, 1984).

CHAPTER 13

INHERITANCE OF QUALITATIVE RESISTANCE TO RACES OF *MELAMPSORA MEDUSAE* IN *POPULUS DELTOIDES*

13.1. INTRODUCTION

Several races of *M. medusae* have been recognised in Australia (Singh and Heather, 1982b) and thus, a cultivar resistant to one race may be susceptible to the other. The inclusion of many races in inheritance studies aids in selection of plants with broad spectrum resistance. Many authors have detailed the importance of using known pathogen races in inheritance studies (e.g. Person and Sidhu, 1971), and the problems arising with bulked inoculum are apparent from the results reported earlier (Chapter 8). Muhlelarsen (1963), following a field study of a population of poplar progeny to field isolates of an unspecified species of *Melampsora*, concluded that resistance was controlled by a few to several dominant genes.

This chapter reports a study of inheritance of resistance to five races of *M. medusae* in a *P. deltoides* (2n=38) cross, assessed on a qualitative scale.

13. 2. MATERIALS AND METHODS

13.2.1 ORIGIN OF HOST PARENTS : The parents *P. deltoides* cvs. 60/122 (female, P1) and T-173 (male; P2) are selections from separate Texan provenances (Appendix 2). The cv. 60/122 is relatively more susceptible than cv. T-173 to all the races.

13.2.2 HYBRIDIZATION : Crosses were performed on bottle grafted branches of cv. 60/122 in a glass house as described by KNOX *et al* (1972). The branches, bearing unopened female flower buds, were collected from the poplar plantation maintained by the Botany Department, Australian

National University, and washed with a jet of tap water. The grafts (Plate 13.1a) were made during winter 1982 (mid August) about 3-4 weeks prior to natural flowering in the field. The understocks (rooted for 4 months), consisting of cuttings 30-40 cm long, and 1.5-2.0 cm in diameter, were transplanted into 20 cm pots. Pollen of cv. T-173, collected from plantations at Kempsey, NSW was dried over silica gel (24 h) and stored in a freezer (-14°C) for 1-2 weeks prior to use. Crosses were conducted, in an isolated, pollen proof glass house during early morning, by gently dusting the pollen on the opening female buds (Plate 13.1b). Several other crosses were performed using separate cultivars but were not included in the study since insufficient progeny were obtained. A separate study of inheritance of resistance in three crosses of *P. deltoides* cultivars, is presented in Appendix 9.

Seeds were collected after 60-70 days from mature capsules (Plate 13.1c) and germinated in Perlite under a mist spray. A nutrient solution was applied twice-weekly, and the seedlings transplanted when the first true leaf pair unfolded (*c.* about three weeks) (Plate 13.1d) into pots containing Perlite and Vermiculite (1:1). About 110 F1 plants were raised from this cross and individually labelled (Plate 13.1e).

The parents were propagated by clonal cuttings under mist and grown along with F1 plants under controlled conditions in a rust free glass house ($20\pm3°$ C, 16 h photoperiod). Liquid fertilizer (Hortico®) was applied once a week and a bacterial insecticide Lane Dipel® HG (*Bacillus thuringienisis Berliner var. alesti*) was sprayed occasionally to control leaf eating caterpillars. Plants were cut back every 3 months to ensure the development of fresh leaves of uniform age suitable for optimal disease expression (Sharma *et al*, 1980). The tests were conducted on leaves from plants aged 12-24 months (Plate 13.1f).

13.2.3 PATHOGEN ISOLATES : Five races of *M. medusae* were employed to study the inheritance of resistance in this cross (Races 1A, 2A, 3A, 4B and 5A) and details on multiplication of the races are presented elsewhere (section 2.3, 2.4 and 2.5). The urediniospore of *M. medusae* is a dikaryote (2n) and is asexually (clonally) propagated. Four of the five races (1A, 2A, 3A and 5A) originated from a single, separate

Plate 13.1

a. The branch a female poplar clone (maintained in the bottle), grafted to another poplar plant in the pot, prior to hybridization.

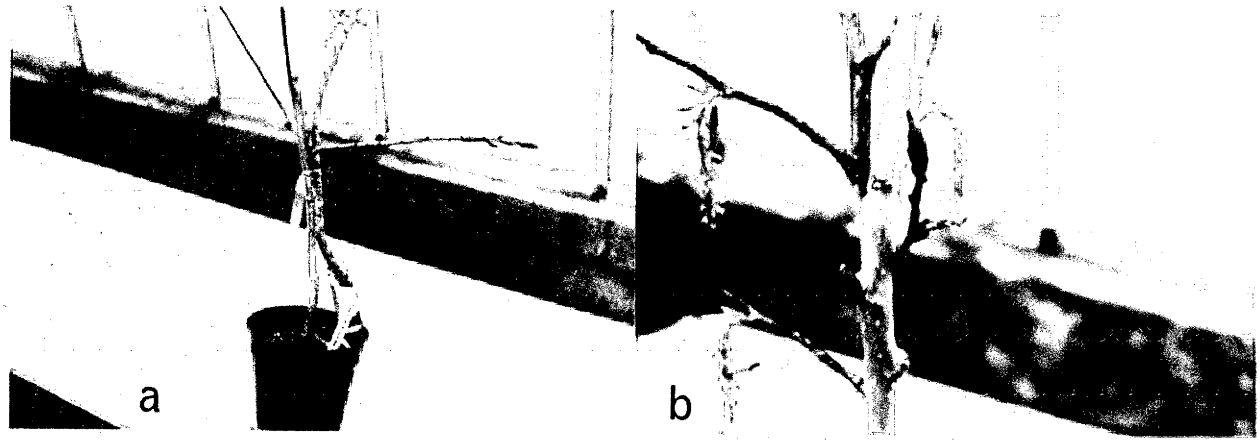
b.The opened female flowers of poplar on a grafted clone.

c. The mature capsule, before dehiscence, of a grafted and pollinated branch of a female clone.

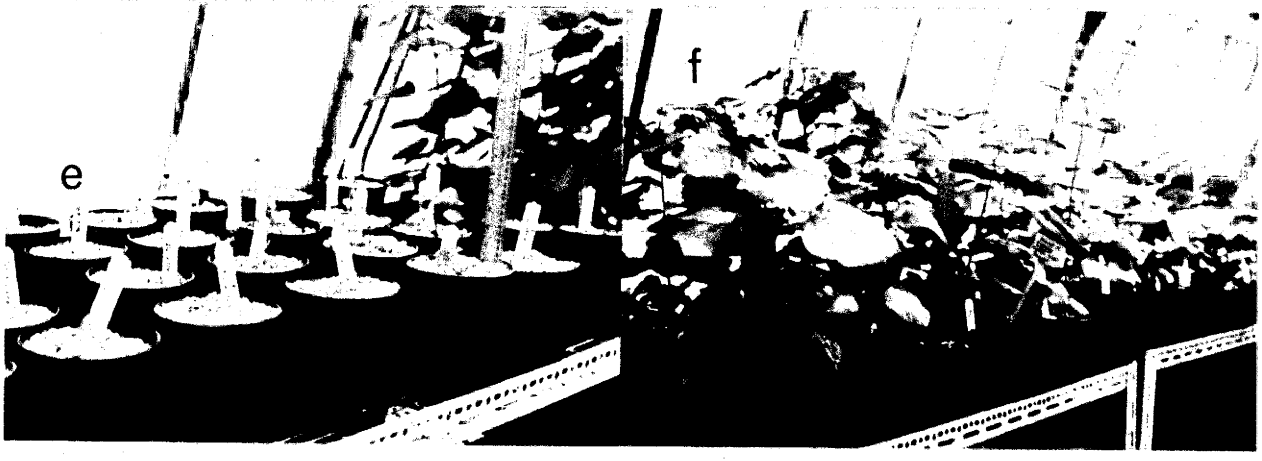
d. Two week old seedlings of the F1 progeny of a manipulated cross of poplar clones.

e. Seedlings after transplanting.

f. Seedlings (c. six months old) maintained in the glass house.


a
b


c
d


e
f

urediniospore (Singh and Heather, 1982) while race 4B was isolated from race 4A for its avirulence on cv. T-173 using a leaf replica technique (Appendix 3).

13. 2.4 INOCULATION : The disease reaction of each plant to each race was assessed by using leaf disks (five replicates) from the individual plants. Because of the large population involved, the tests were conducted in three batches (Races a) 1A and 2A, b) 3A, c) 4B and 5A were assesed separately).

Leaf disks (1.70 cm^2) were punched from freshly harvested, surface sterilised leaves of uniform age (c. 3 months). Due to the segregation in the F1 population, minor differences in leaf maturity were noticed; they were insufficient to influence the relative variation of disease expression. Again, due to replication of leaf disks in the inoculation, it was possible to assess only c. 60 randomly selected, F1 plants for reaction to a particular race pair.

All the leaf disks of F_1 plants and the parents were inoculated separately with the urediniospores of individual races using a spore settling tower (section 2.4.2). Replicate leaf disks (5 for F1 plants, 10 for each parent) from individual plants, some cover glasses and several disks of cv. I-488 were randomly placed in the bottom of a spore settling tower and inoculated in three successive batches, with 5 mg of fresh spores of a race. Subsequently, separate replicate disks and cover glasses were inoculated with spores of the second race of the pair. Comparability of deposition between inoculations, within and between races, and germination (> 95% for all races) were checked by examining the coverglasses, while the uniformity of infection was checked on the disks of the universal suscept, cv. I-488. Inoculated leaf disks were placed on plastic foam soaked with GA solution (10 mg/L) sealed in glass Petri dishes and incubated in control growth cabinets (standard environment; section 2.5.2).

13.2.5 DISEASE ASSESSMENT :The level of disease in each plant was assessed as Infection Type (IT) on day 18 and confirmed at day 23 of incubation. The IT was on a 0 to 4 scale of increasing disease severity, where 0 - immune, no macroscopic symptoms of disease, 1 -

chlorotic/necrotic symptoms with possibly one or two minute uredinia, 2 to 4 -increasing size and number of uredinia (section 2.6.1, Plate 2.3). For the computation of the genetic ratios, IT 0 and 1 in the plants were classed as resistant and 2 - 4 as susceptible reactions. This classification distinguished among the plants those on which the rust reproduced (IT 2-4), and those on which there was little or no reproduction of the pathogen (IT 0-1). The ratios (susceptible : resistant) were tested for goodness of fit to several hypothetical genetic ratios using chi-square test and those ratios which gave the best fit i.e. the highest probability range, were accepted.

13.3 RESULTS

While for all races, cv. 60/122 (P1, female) was consistently more susceptible than cv. T-173 (P2, male), the specific reaction of the parents was dependent on the race employed (Table 13.1) and thus the cross could be classifed as Resistant X Resistant, Susceptible X Resistant or Susceptible X Susceptible (Table 13.1). With all the races, the F1 plants exibited clear segregation indicating the heterozygosity of the parents. All the races elicited different ratios in the progeny (Table 13.1) thus confirming their status as distinct races (sensu Stakman and Christensen, 1953), which had been recognised by their reaction on differential cultivars (section 2.3.1). The reaction of individual F1 plants to these races has been provided in Appendix 8.

Races 1A, 2A and 3A elicited a Susceptible (60/122) X Resistant (T-173) reaction in the parents. While reaction to race 1A in the progeny approximated a 1:1 ratio, suggesting the role of one gene (dominant or recessive, L or l) for resistance (P=0.5-0.8), race 2A elicited a 3:1 (S:R) ratio (P=0.8-0.9) indicating that resistance in cv. T-173 was conditioned by the concurrent occurrence of two, homozygous, recessive genes to this race (qqmm) and cv. 60/122 was dominant heterozygous for both the genes (QqMm). However the reaction

Table 13. 1 : Reaction of parents P. deltoides cvs. T-173 and 60/122 and their F_1 hybrids to five races of M. medusae and the X^2 probability levels of certain genetic ratios for segregation in the F_1 progeny.

RACE	INFECTION TYPE AND RESISTANCE CLASSIFICATION PARENTAL CVS.		NUMBER OF F_1 PROGENY					RATIO OF RESISTANT TO SUSCEPTIBLE IN PROGENY		PROBABILITY RANGE (X^2)	PROPOSED GENETIC MAKE-UP OF PARENTS
			0	1	2	3	4	OBSVR		FITTED	
	60/122	T-173	R				S	R : S			60/122 X T-173
1A	3 (S)	1 (R)	0	27	1	15	16	27:32	1:1	0.5-0.8	ll x Ll
2A	3 (S)	1 (R)	0	14	1	16	28	14:45	1:3	0.8-0.9	QqMm x qqmm
3A	3 (S)	1 (R)	5	17	24	11	7	-	-	-	QUANTITATIVE
4B	1 (R)	0 (R)	27	28	7	4	2	55:13	3:1	0.25-0.5	Uu X Uu
5A	1 (R)	0 (R)	18	32	13	1	4	50:18	3:1	0.7-0.8	Vv X Vv

† R and S refer to Resistant and Susceptible reactions, respectively

to race 3A by the F1 plants was less discrete, the classes approximating a normal distribution and thus the resistance to this race may be additively inherited and possibly controlled by three or four genes.

Races 4B and 5A were avirulent on both the parents and the progeny reaction approaches a 3:1 (R:S) ratio (P= 0.25-0.5 and 0.7-0.8 respectively) suggesting the action of a single, dominant gene for resistance to each of these races in each of the cultivars (U and V respectively). Most of the F1 plants resistant to race 5A were also resistant to race 4B suggesting possible linkage of these two resistant genes. As there were certain plants which were resistant to one race but susceptible to another, the two genes are likely to be separate (Appendix 8).

Many of the F1 plants (and the male parent, cv. T-173 to race 4A) exhibited degrees of 'slow rusting', characterised by a long latent period and reduced sporulation, suggesting the presence of modifying factors influencing the disease expression in these cultivars. While such characteristics did not affect the classification of individual plants as resistant (IT 0 or 1) or susceptible (IT 2-4), but depending on the period after inoculation when disease severity was assessed, they influenced the grading of plants within the IT 2-4 range.

13.4 DISCUSSION

The dioecious nature and long generation interval (7-8 years) of the host and the absence from Australia of the sexual stage of the pathogen make genetic studies in this system difficult. Thus hybridization amongst the pathogen races and selfing of the host clones, is not possible. Also, backcrossing and further generation testing in poplars are difficult and time consuming. Hence, the genetic interpretation of the results on inheritance of leaf rust resistance in poplars is largely hypothetical. However, since genetic studies have not been reported in this system, any information would assist in determining breeding strategy. The strict control of the environmental variables (both pre- and post-inoculation conditions of plant culture, and unifomity of leaf

maturity and inoculum) resulted in considerable uniformity in disease expression in the replicate leaf disks of an individual plant. This ensured that the variation between individuals in infection type was genetically based.

The disease reaction (basis IT) of the parents and the ratios of the progeny were clearly dependent on the race of M. medusae employed emphasising the importance of employing distinct races in disease resistance studies (Person and Sidhu, 1971). In these clones of P. deltoides resistance appears to be dominant with races 1A, 4B and 5A, but recessive with race 2A, and codominant or additive to race 3A (Table 13.1). It can be hypothesised that resistance/susceptibility to M. medusae was monogenic (races 1A, 4B and 5A), controlled by two complementary genes (race 2A), or three to four additive genes (Race 3A) depending on the race employed. Muhlelarsen (1963), analysing the progeny of several poplar crosses involving P. deltoides and P. nigra under field conditions, concluded that resistance to Melampsora spp. was controlled by one or two dominant complementary or duplicate genes and this agrees with some, but not all, of the present results.

Almost all the F1 plants showed a high level of racial specificity and this may result from the different resistance genes they contained. Such genotypes could still be useful in mixed clonal plantings similar to the multiline approach suggested by Heather and Chandrashekrar (1982).

The results for races 2A and 3A contrast with those frequently reported in inheritance studies in agricultural crop plants where resistance is predominantly monogenic dominant (Person and Sidhu, 1971). However as Wolfe and Schwarzbach (1978) and Day et al (1983) have suggested, the latter patterns of resistance may be artefacts of breeding strategy, which has concentrated on few resistance genes each with a large effect. Thus, in a forest plant like poplar, where little or no breeding for resistance to leaf rust has been practised, the observation of a multiple gene or recessive gene control is not unexpected.

Some plants exhibited a relatively long latent period; possibly certain modifying factors are influencing disease expression and resulting in 'slow rusting' of these susceptible plants. However,

for more precise assessment of such resistance in these plants, it is necessary to inoculate them with races virulent on both parent cultivars and make quantitative measurement of the disease traits; this is the subject of some further work (Chapter 14).

13.5 SUMMARY

The F1 progeny of a P. deltoides cross were analysed independently for their qualitative reaction to five races of M. medusae which, depending on race employed, elicited susceptible X resistant, resistant X resistant or susceptible X susceptible reaction in parents. Resistance was inherited as dominant (3 races), recessive (1 race) or additive (1 race) in the clones, and was controlled by a single gene or two genes acting in a complementary manner. Modifying factors influenced the degree of susceptiblity expressed in certain plants and resulted in a 'slow rusting' effect.

CHAPTER 14

STUDIES ON INHERITANCE OF QUANTITATIVE RESISTANCE TO *MELAMPSORA MEDUSAE* IN A CROSS OF *POPULUS DELTOIDES* CLONES

14.1 INTRODUCTION

Inheritance of qualitative resistance in poplars to *M. medusae*, characterised by complete resistance or susceptibility of the plants to the races employed, was reported in Chapter 13. However, there was evidence in some progeny and in cv. T-173 of occurrence of quantitative resistance to certain races. Analysis of the inheritance of quantitative resistance in the host, which parallels the aggressiveness in the pathogen would be useful. The merits of such resistance, which is essentially a partial resistance (cf. Parlevliet, 1979), often characterised by 'slow rusting' (*sensu* longer latent period; cf. Wilcoxson, 1981) has been discussed in the literature (e.g. Vanderplank, 1968; Nelson, 1978; Parlevliet, 1979 and 1983 a and b; Wilcoxson, 1981; Johnson, 1983 and 1984), and has been summarised in the introduction to this section (Chapter 12).

Quantitative resistance is primarily a lower degree of susceptibility, marked by lower rate of disease expression and disease severity, as opposed to complete or qualitative resistance which is usually characterised by a hypersensitive type reaction. Although many terminologies describe such quantitative resistance (e.g. polygenic resistance, non-specific resistance, horizontal resitance, rate-reducing resistance, partial resistance, incomplete resistance etc.), the present terminology has been adopted as it does not imply any genetic or 'race non-specificty' connotations. Quantitative resistance may be oligo or polygenic, specific or non-specific, durable or not durable, it merely implies resistance as measured by quantitative traits of the disease. The term 'slow-rusting' is used, as a synonym of quantitative

resistance, with emphasis on a longer latent period.

Thus, by definition, quantitative resistance implies the presence of some disease, and genetic analysis of such resistance can be best done with races virulent on both parents. Clifford (1974) and Parlevliet (1980a) have discussed the problems of hypersensitive genes masking the potential susceptibility of the genotype, where true levels of the disease, when the host is attacked by a virulent race, cannot be easily predicted. Further, studies reported in Section I (Chapters 5, 6, 7 and 8) illustrate that host-pathogen specificity (at the cultivar-race level) can occur also at a quantitative level between components of resistance in the host and aggressiveness traits in the pathogen. Similar specificity (physiologic specialisation) between host and pathogens for quantitative traits have been observed in other systems (e.g. Clifford and Clothier, 1974; Johnson and Taylor, 1976; Parlevliet, 1977; Wilcoxson, 1981) and thus it is may be useful to employ more than one race for selection of such resistance.

In preliminary studies, the race 5M (designated as 5M2 in chapters 5 and 6) was virulent on cvs 60/122 and T-173. Race 7A, which had been isolated earlier by the author in natural stands of cv. 60/122 (section 2.3.1), was also virulent on both of these cultivars. The present study was designed to test the quantitative reaction of the F1 progeny of the cross P. deltoides cv. 60/122 (female) X cv. T-173 (male) (see Chapter 13) with races 5M and 7A, to obtain genetic information on inheritance of resistance traits of rate and severity of disease, and for possible selection of some clones with higher quantitative resistance.

14.2 MATERIALS AND METHODS

14.2.1 SOURCE OF PARENTS, HYBRIDIZATION TECHNIQUES AND CULTURE OF PLANTS : The F1 progeny (61 plants) of the cross cv. 60/122 X cv. T-173 were the same as those used in the previous study (Chapter 13). Clonal plants of the parents and F1 were raised in a rust-free glass house under controlled conditions and details on culture of plants have been

described earlier (section 2.2 and Chapter 13).

14.2.2 THE PATHOGEN RACES : The races 5M and 7A of M. medusae employed in this study were multiplied on detached leaves of cv. I-488, and fresh urediniospores thus obtained were subsequently dried (section 2.4 and 2.5) and used immediately for inoculation.

14.2.3 INOCULATION AND INCUBATION : The disease reaction of each plant to each race was assessed by using five replicate leaf disks from the individual F1 plants and ten leaf disks of the parents.

Urediniospores (4 mg) each race (5M and 7A) were deposited separately on replicate leaf disks of 61 F1 plants of cross cvs 60/122 X T-173, and of the parents, using a spore settling tower (section 2.4.2). Due to the large number of disks, multiple inoculations were necessary. However, differences in number of urediniospores between and within inoculations were less than 5%, and germination of the urediniospores was more than 95% in both races, as assessed on the cover glasses included in each inoculation (section 2.4.2).

Inoculated leaf disks were sealed in glass Petri dishes containing foam soaked with GA (10 mg/L), and incubated under 'standard environment' in control growth cabinets (section 2.5).

14.2.4 OBSERVATIONS ON DISESAE TIMING AND DISEASE SEVERITY : Five traits of quantitative disease expression viz. incubation period to flecking (IPF), latent period to production of first uredinium (LP1), uredinia produced per leaf disk (ULD), urediniospores produced per square mm of leaf area (USM) and urediniospores produced per uredinium (USU) were measured on the replicate leaf disks of the progeny and parents. Longer IPF and LP1 and smaller ULD, USM and USU indicate higher resistance.

14.2.5. STATISTICAL ANALYSIS AND TECHNQIUES : Data were initially tested for homoscedasticity and normality of error variances (section 2.7.1) and transformed where necessary. The transformed data (for ULD, USM and USU) were used for all subsequent analyses. The analysis of variance (between 2 races and 61 plants) was conducted, and the parental mean, midparent values, F1 mean and range computed for all traits. The

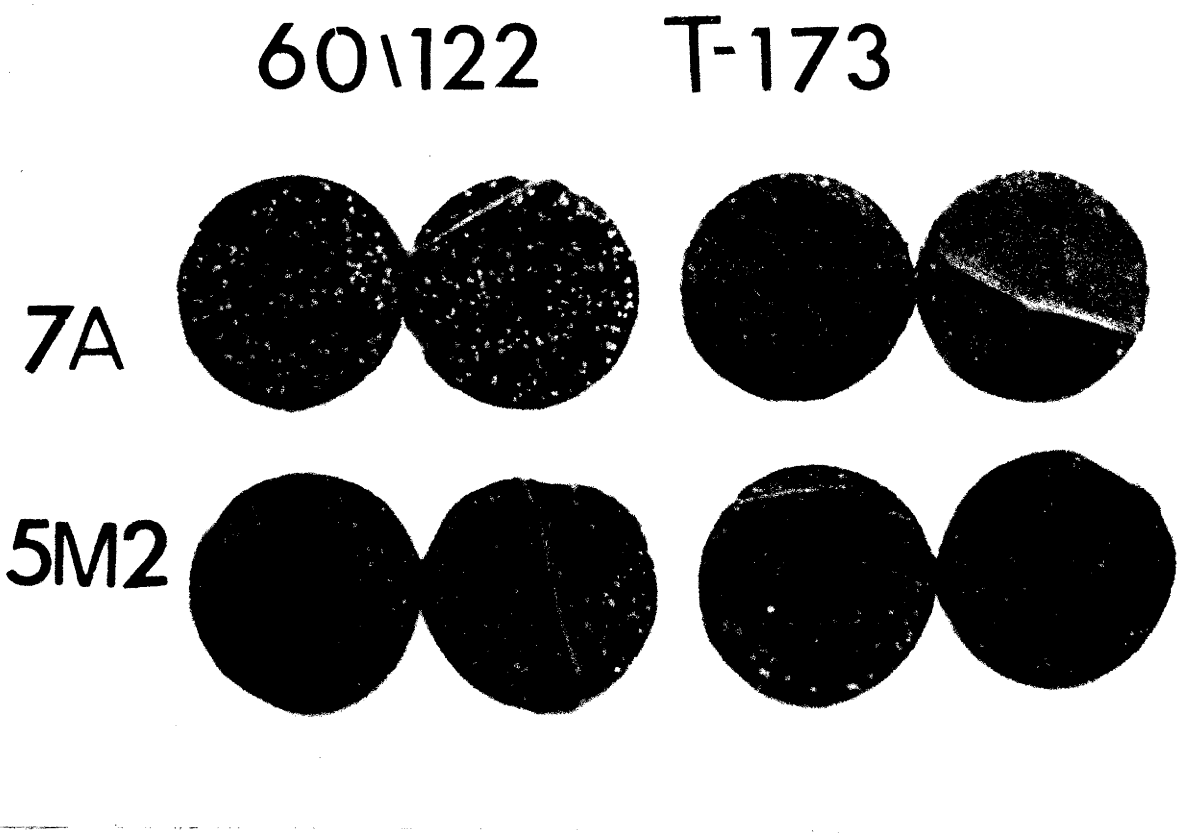


Plate 14.1 The differing aggressiveness of M. medusae race 7A and race 5M2 (5M) of M. medusae on P. deltoides cultivars 60/122 and T-173 (Parents 1 and 2 respectively).

frequency graphs were constructed for each trait using the progeny means. The test for goodness of fit of the distribution of the progeny (mean values) to the normal distribution was conducted using the MPL statistical program on a UNIVAC computer (Ross, 1980) adopting the class interval used in the graphs. The goodness of fit to the normal distribtution was tested using the chi-square values. Skewness and kurtosis for the observed distribution among the F1 plants were estimated using the MPL program, and significance of skewness was tested by dividing the mean by standard error and comparing with Z values at 0.05 level (1.96).

The heritability (broad sense) (h^2) estimates were made for each trait from the analysis of variance wherein the variance due to progeny X race interaction was added to the error variance (Allard, 1960) and h^2 was computed by dividing the genotypic variance by the phenotypic variance. Phenotypic variance was the Mean Sum of Squares (MSS) for the Progeny (total variance due to differences in the reaction of the F1 plants) and genotypic variance was

Phenotypic variance - error MSS/no. of replications (10).

The heritability was also estimated for reaction to each race using the above equation. Correlation coefficients between the traits were computed using the cell mean values with a GENSTAT program (section 2.7).

14.3 RESULTS

14.3.1 ANALYSIS OF VARIANCE: The analysis of variance for five traits of disease induced in 61 F1 plants of the cross 60/122 X T-173 by two races of M. medusae are presented in Table 14.1. The contribution to the variation observed in all traits by the F1 plant, race and their interaction were highly significant ($P < 0.001$). The F1 plant was by far the highest contributor to the variation (except IPF where race was the most important contributor), race a lesser contributor, while the plant X race interaction was least important cause of such variation, in all traits (Table 14.1).

Tables 14.1 Analysis of variance[a] for five disease traits[b] by the F_1 progeny of the P. deltoides cross cvs. 60/122 X T-173 with two races of M. medusae

Source	D F	IPF	LP1	ULD	USM	USU
F_1 Plant	60	4.59	20.97	21.24	10.10	2.19
Race	1	6.50	6.47	4.25	1.47	0.41
Plant X Race	60	1.10	5.62	3.76	1.04	0.56
Residual	488	0.05	0.16	0.36	0.06	0.23
Total	609	0.62	2.76	2.76	1.15	0.45

[a] Mean sum of sqaures presented; All values are highly significant ($P < 0.001$).

[b] The five traits are incubation period to flekcing (IPF), latent period to production of first uredinium (LP1), uredinia produced per leaf disk (ULD; square root transformed), urediniospores produced per sq. mm (USM; $\log_e$ +1 transformed), and urediniospores produced per uredinium (USU, $\log_e$ + 1 transformed).

When the variance due to the interaction is added to that of residual, the main effects remained significant ($P < 0.001$), except for race in USU. However, the significant interaction component indicates that the level of disease on a specific F1 plant varied with the race employed; and thus for all traits a degree of physiologic specialisation by these races on the plants studied, was evident.

14.3.2 DISEASE REACTION OF PARENTS TO TWO RACES : The mean for both parents and mid parent values, with two races are presented in Table 14.2. The female parent cv. 60/122 was more susceptible than male cv. T-173 to both races, and the latter was characterised by a slower rate of disease initiation (longer IPF and LP1) and lower disease severity (smaller ULD, USM and USU) (Plate 14.1). However between the parents, for most traits, there were clear differences in the ranking of the races for aggressiveness (Plate 14.1). On cv. T-173, Race 5M was relatively more aggressive than race 7A while on cv. 60/122, race 7A was more aggressive than race 5M for all traits except IPF.

14.3.3 DISEASE REACTION OF THE F1 PLANTS TO TWO RACES : The mean (population), standard deviation, range observed, skewness and kurtosis of the distribution, along with significance of deviation from normal distribution, of the F1 plants for five traits of disease observed for both races are presented in table 14.2. The observed frequency distribution of the F1 plants along with parental positions for five traits are presented in Fig. 14.1. As some features of the F1 plants were similar with both races, in the following presentation, the particular race is specified only when there are marked differences between the reaction of two races. Also, for ULD, USM and USU, the transformed values are presented and thus, the differences between treatments at the higher level would appear smaller than those actually observed.

There was a clear segregation of the F1plants for all disease traits (Fig. 14.1) indicating the heterozygosity of the parental clones used. Also, for all disease traits, the range of mean values among F1 plants exceeded both the parental means (Table 14.2, Fig. 14.1) indicating the transgressive segregation in the progeny of this cross.

Table 14.2 Means of parents[a], midparent values, mean of the F_1 population[ab], range of F_1 values[b], skewness[c], kurtosis and significance of fit of the F_1 distribution to the normal, for the P. deltoides cross cvs 60/122 X T-173 for five traits of disease to races 5M and 7A of M. medusae

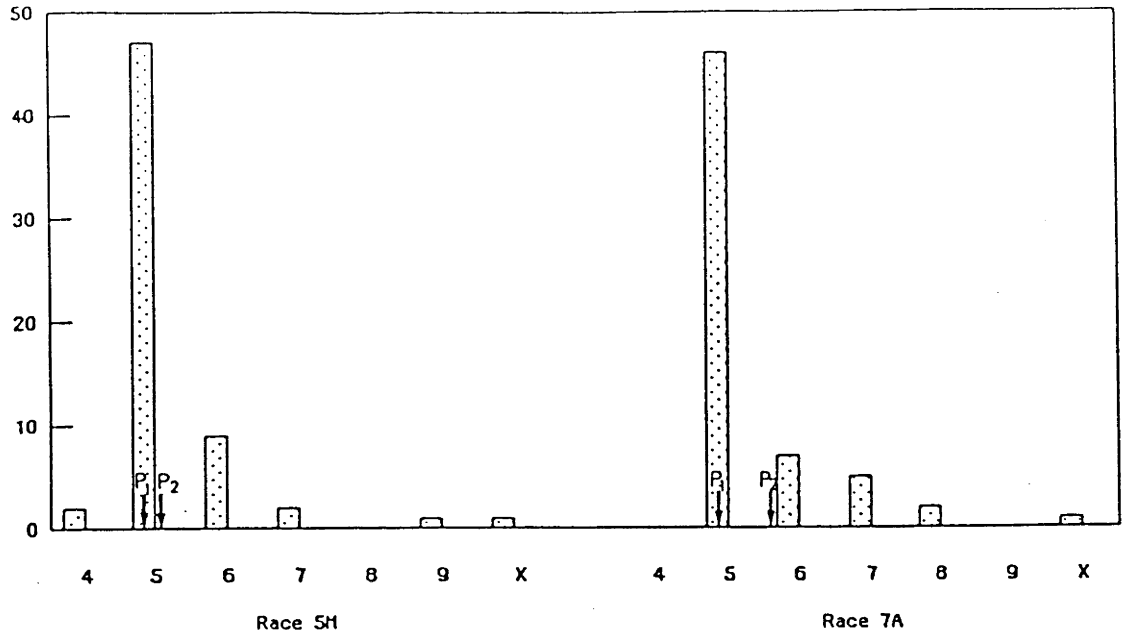
Trait	P_1 60/122	P_2 T-173	Mid Parent Values	F_1 Mean	Range of F_1 Values	S.D.[d] for F_1	Skewness	Kurtosis	Sign. of fit to normal (P)	L.S.D (0.05)[e]
a) **WITH RACE 5M:**										
IPF	5.00	5.20	5.10	5.24	4.20-9.00	0.67	3.31	13.89	<0.001	0.290
LP1	8.60	10.80	9.70	9.74	7.40-15.40	1.47	1.25	2.16	<0.01	0.504
ULD	6.56	5.95	6.25	6.43	1.89-8.79	1.55	-1.57	3.85	0.25-0.75	0.748
USM	6.81	6.33	6.57	6.33	2.55-7.31	1.08	-3.95	18.62	<0.01	0.313
USU	10.87	10.59	10.73	10.54	9.88-11.51	0.56	-1.58	7.70	0.25-0.75	0.601
b) **WITH RACE 7A**										
IPF	5.20	5.80	5.40	5.44	4.60-8.00	0.82	1.84	2.39	<0.001	0.290
LP1	8.00	12.20	10.10	9.94	7.60-15.40	1.61	1.19	1.48	<0.01	0.504
ULD	7.67	5.48	6.58	6.59	3.31-9.70	1.62	-1.01	3.01	0.1-0.25	0.748
USM	7.15	6.14	6.65	6.41	4.61-7.51	1.01	-4.25	24.01	<0.05	0.313
USU	10.89	10.57	10.73	10.59	9.52-11.75	0.49	0.33	-0.03	0.90-0.95	0.601

Symbols for five traits of disease are as presented in Table 14.1; [a] Each value is a mean of ten replicates for parents and five replicates for F_1 plants. [b] A F_1 plant which was completely resistant was ignored in computing the range (plant 81 in Appendix 8).

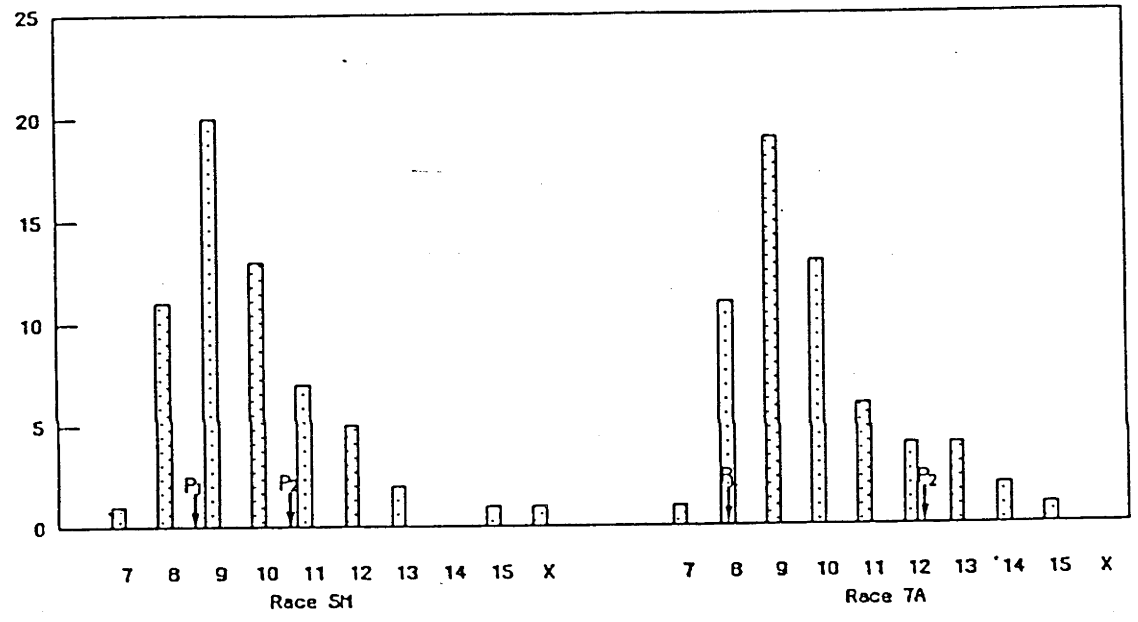
[c] Skewness of distribution of all five traits with both races are significant ($P < 0.05$).

[d] Standard deviation of mean of the population. [e] Least Significant Differences (LSD) computed with the interaction component of both races on the F_1 plants, and thus the same values for both races.

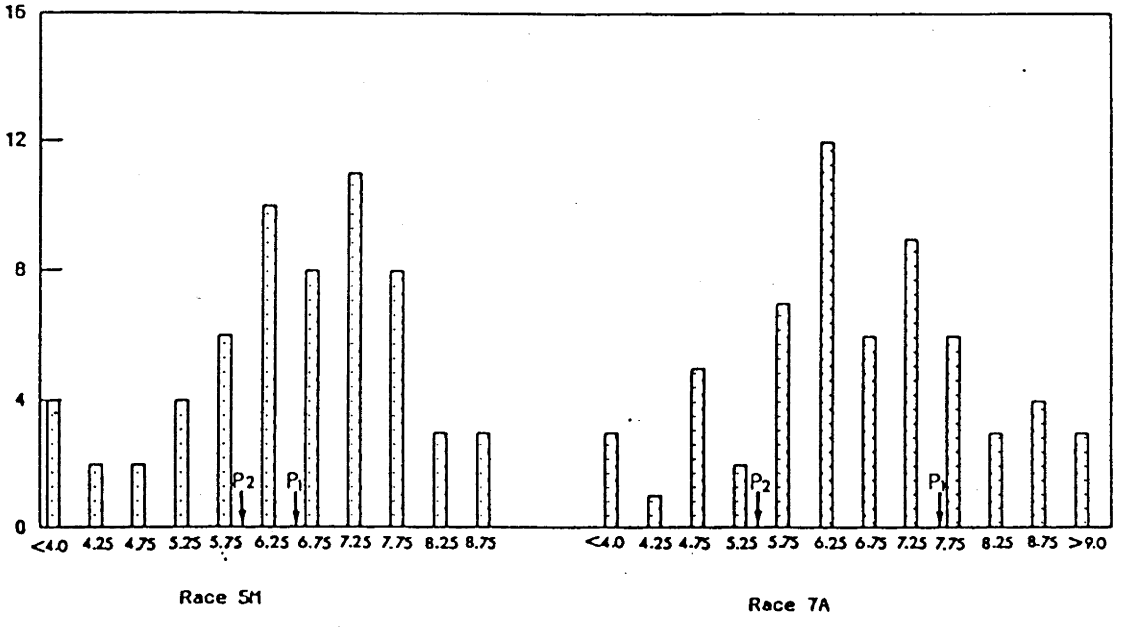
Figure 14.1 The frequency distribution of reaction of the F1 plants of the cross P. deltoides cvs 60/122 X T-173, to races 5M and 7A of M. medusae, for five disease traits. Arrows for P1 and P2 show the relative reactions of cvs 60/122 and T-173, respectively.



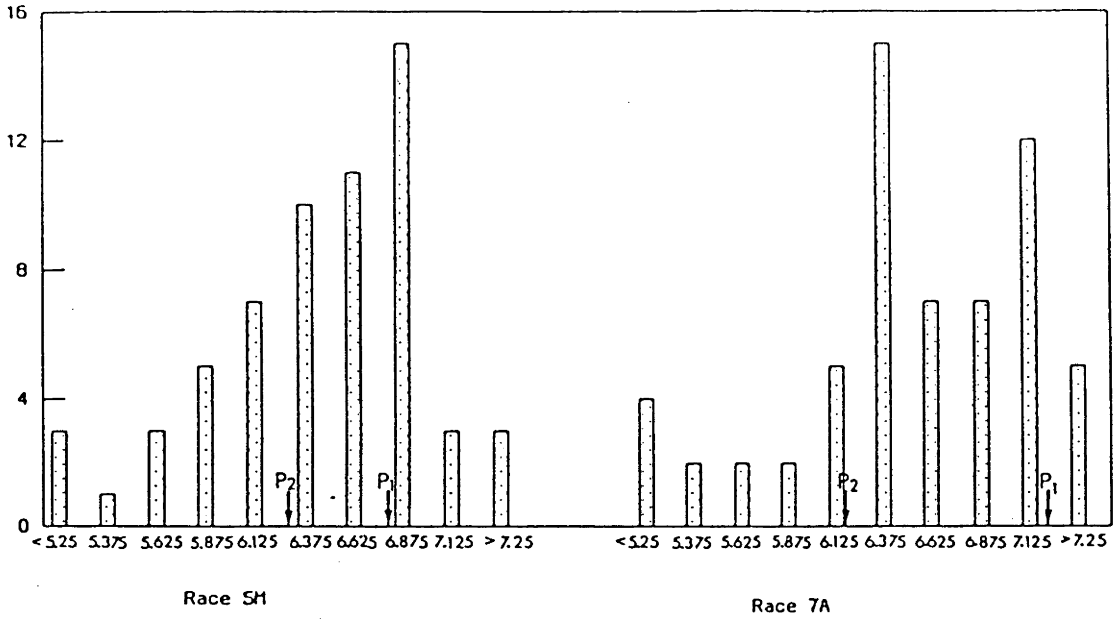
a. Incubation Period to Flecking (Days)



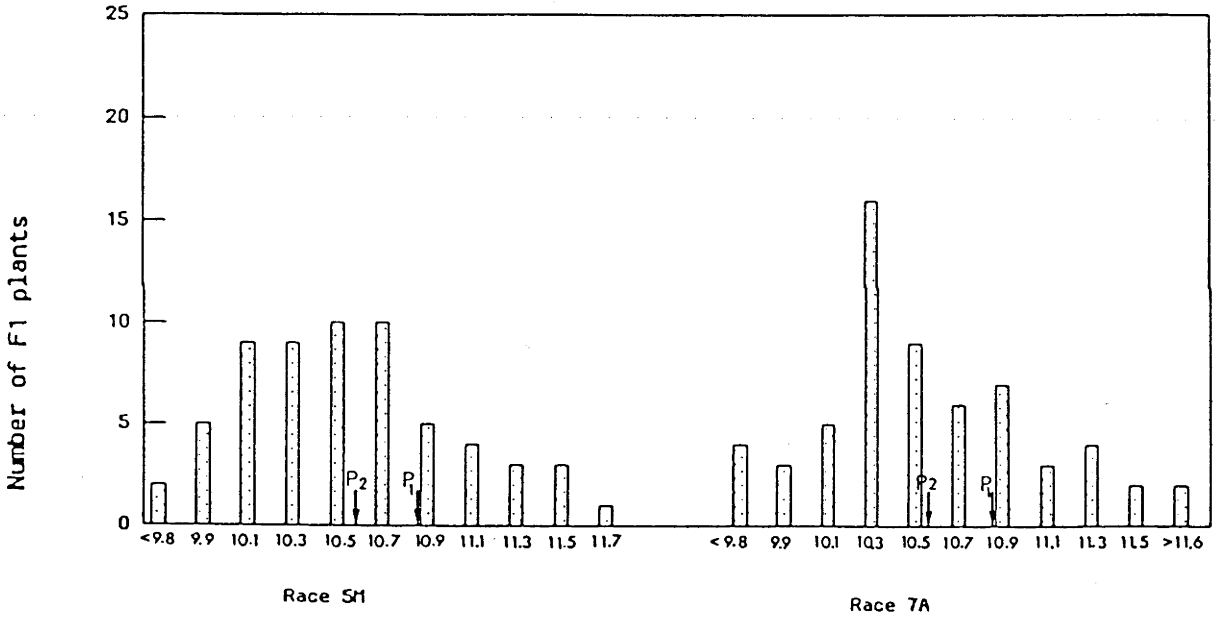
b. Latent Period to First Uredinium (Days)



C. Uredinia Produced Per Leaf Disk (sq. root transformed)



d. Urediniospores Produced per sq mm of leaf (loge+1 transformed)



e. Urediniospores Produced per Uredinia (loge+1 transform)

Table 14.3 Matrix of correlation coefficients (r)[a] between the five traits of disease among the F_1 plants of the cross 60/122 X T-173 to races 5M and 7A of M. medusae. (D F = 60).

a) **WITH RACE 5M**

	IPF	LP1	ULD	USM	USU
IPF	1.00				
LP1	0.35	1.00			
ULD	-0.37	-0.68	1.00		
USM	-0.47	-0.71	0.65	1.00	
USU	-0.37	-0.41	0.08^{ns}	0.81	1.00

b) **WITH RACE 7A**

	IPF	LP1	ULD	USM	USU
IPF	1.00				
LP1	0.47	1.00			
ULD	-0.42	-0.67	1.00		
USM	-0.35	-0.79	0.59	1.00	
USU	-0.07^{ns}	-0.38	-0.10^{*}	0.72	1.00

[a] All unmarked values are highly significant ($P < 0.001$).

^{ns} denotes non significant ($P > 0.25$), while * denotes significant at P = 0.75-0.90.

Further, for all traits (except USU with race 7A), the fit to normal distribution was low to moderate (P, 0.001 to 0.75). For all traits, the skewness was significant ($P < 0.05$), and was positive for IPF and LP1, but negative for ULD, USM and USU. This indicates the occurrence of dominance or non-additive interaction in this cross, with higher resistance being recessive to lower resistance for all traits.

With race 5M, the parents differed little for IPF, but with race 7M the differences were more pronounced (Table 14.2), and thus the concentration of the progeny around the parental values for IPF (Fig. 14.1a) is not surprising. Although there was a transgressive segregation towards longer IPF, very few plants with IPF shorter than the shortest parent (cv. 60/122) were observed.

Although the latent period (LP1) measures the day to production of first uredinium, usually 20-30% of the uredinia appear together on this day. The distribution of the F1 plants for latent period (LP1) was relatively more continuous (Fig. 14.2b). Whilst the modal class was closer to the shorter LP1 parent (cv. 60/122), the tail towards longer LP1 was pronounced and was beyond the values of the longer LP1 parent (cv. T-173). This may have resulted in the mean F1 values being similar to mid parent values for both races (Table 14.2).

The frequency distribution of the F1 plants for uredinia produced per leaf disk (ULD) had a relatively better fit to normal than that of other traits (Table 14.2, Fig. 14.1c). Skewness was negative but significant, ie. towards the high ULD (more susceptible) parent (cv. 60/122). Considerable transgressive segregation beyond the susceptible parent was observed for this trait, particularly with race 5M.

The F1 mean values, for urediniospores produced per sq. mm (USM), were closer to that of the resistant parent (Table 14.2) and also, the transgressive segregation beyond this parent was considerable (Fig. 14.1d). With both races, there was a clear lack of fit of the F1 plants to normal distribution with a considerable peak (and thus the high kurtosis values) near the susceptible parent (Table 14.2, Fig. 14.c).

For urediniospores produced per uredinium (USU), the

distribution of the F1 plants was normal, with a particularly good fit for race 7A (Table 14.2e). Although the differences between the parental mean values were less, the F1 mean values were nearly equal to that of the resistant parent (T-173) indicating the partial dominance of low USU over high USU. The Skewness was positive with race 5M but negative with race 7A, suggesting the differences in the races corresponding to the ability of plants to support sporulation. However transgressive segregation beyond both parents was evident with both races.

Although there was similarity in the reaction by the F1 plants to both races, there was also significant differences in the racial specificity of the plants. For example, some plants which were moderately susceptible for a given trait to race 5M were highly susceptible to race 7A, and vice versa; such differences would be responsible for the significant plant X race interaction seen in ANOVA (Table 14.1). (Note : the infection type reaction, to races 5M and 7A along with those races referred in Chapter 13, of all the F1 plants used in the study is separately presented in Appendix 8).

While all the F1 plants were susceptible in varying degrees to both races there was one plant (plant no. 81 in Appendix 8) which was completely resistant to both races and was not included in the F1 range presented in Table 14.2. This suggests that both of these races carry an avirulent gene corresponding to a resistant, probably recessive, gene in this cross. However the same plant was highly susceptible to many other races of M. medusae (Chapter 13; Appendix 8), suggesting that the high resistance in this plant to these two races may not be due to the cumulative effect of many resistance genes.

16.3.4 HERITABILITY OF RESISTANCE TRAITS : The broad-sense heritability estimates*(fixed race effect) for all traits were 96-98% but for USU it was 90%. When heritability values were computed separately with each race, they increased by some 1-2% for all traits but for USU they were 82 and 83% with races 5M and 7A respectively. The high heritability values indicate that the interaction of environmental variance in the present study was very low and thus most observed variation was due to

*Alternate heritability values using Allard (1960; pp 96-98) may be slightly lower. e.g. for LP1 - 87% and USU - 82%.

genetic differences between the plants. The relatively lower heritability estimates when race effects were included are due to the presence of plant X race interactive variance.

16.3.5 THE CORRELATION BETWEEN TRAITS OF DISEASE : The matrix of correlation coefficients (r) between different traits of disease in the F1 plants with race 5M and 7A are presented in Table 14.3. Trends of association among traits with both races were generally similar. A positive and highly significant ($P < 0.001$) correlation between IPF and LP1, ULD and USM, USM and USU were observed with both races (Table 14.3). A negative and highly significant ($P < 0.001$) correlation between IPF and all other traits (except with USU for race 5M, ns; $P > 0.25$), and between LP1 and all other traits, were observed. Correlation between ULD and USU was not significant, although it was negative for race 7A and positive for race 5M. The most significant correlation for race 5M was between USM and USU followed by LP1 and USM, and LP1 and ULD; and for for race 7A, it was between LP1 and USM, followed by USM and USU, and LP1 and ULD.

14.4 DISCUSSION

Poplars are dioecious and hence, high heterozygosity among the parental clones could be expected and the segregation observed at the F1 level in the plants for all traits studied, is not surprising. Similar segregation for qualitative resistance was also observed with many races in the previous study (Chapter 13) and for quantitative resistance in other crosses (Appendix 9). Among the parents, cv. 60/122 (P1) was more susceptible than cv. T-173 (lower rate of disease progress, larger number of uredinia, higher sporulation). However, cv. 60/122 was less susceptible than many other poplar cultivars studied (Section I) (e.g. cvs I-154, I-488, W-79/307), and thus it is possible that both cultivars carry numerous genes for resistance, although cultivar T-173 is expected to carry relatively more genes than cv. 60/122 for the resistance traits investigated. However, for IPF and USU, due to the small differences between the parents, it is possible that both parents carry the same genes for these two traits. Race 5M was more aggressive than race 7A on

cv. T-173 while the reverse was the case on cv. 60/122, i.e. the races were most aggressive on the cultivars on which they were initially isolated. Such quantitative specificity of the races on their 'own' cultivars supports earlier observations (Chapter 7 and 8).

For all traits, the distribution of the F1 plants was continuous for all traits rather than discrete suggesting that these resistance traits are polygenically inherited (genes with minor effects). The significant skewness for all traits indicate the presence of non-additive interactive interaction. For certain traits (e.g. LP1), the F1 mean approximated the mid parent values suggesting additive interactions in these traits. The occurrence of transgressive segregation for all traits also supports the presence of additive and non-additive interactions in these traits. Based on midparent values, susceptibility appears to be dominant for IPF and ULD, while resistance seems to be dominant for USM and USU. However, based on the direction of skewness, susceptibility is dominant for all traits, except for USU with race 7A. Due to the heterozygosity of the parents, skewness should be a better indicator than mid parent values of dominance. Many authors have reported the recessiveness of quantitative resistance in other systems (Luke et al, 1975; Parlevliet, 1976; Skovmand et al, 1978; Wilcoxson, 1981; Eskes and Carvalho, 1983; Prakash and Shivashankar, 1984).

For IPF and LP1, although skewness was positive and towards the susceptible parent, plants with IPF shorter than 4 days or LP1 shorter than 7 days were rarely noticed. Parlevliet (1976) made similar observations on the latent period of cultivars of barley against Puccinia hordei. However, IPF or LP1 shorter than those on F_1 plants were not observed on any of the other twenty poplar clones studied earlier (Section I). Thus the physiological barrier may be an important cause of such an observation, i.e. certain minimum time may be necessary for the germtubes of urediniospores to penetrate and establish on the host before subsequent macroscopic expression of disease.

Due to transgressive segregation, the appearance of many plants with long latent periods for both races, is significant from the view of selecting 'slow rusting' plants. Further, increased latent

period was significantly and negatively associated with decreased uredinial number and sporulation, and thus the selection for longer latent period, which can be done with relative ease and accuracy, should be a better criterion in selection for higher quantitative resistance. Latent period has been observed to be signficantly negatively correlated with traits of disease severity in other systems (Parlevliet, 1979; Kulkarni and Chopra, 1980; Wilcoxson, 1981; Eskes, 1983a). Based on principal component analysis of these plants for all traits, LP1 had the highest loading on the latent vectors, suggesting the reasonable selection for other traits based on selection of longer LP1 (details not presented). While the observations agree with that of Parlevliet (1977) on barley leaf rust, the association could be mainly due to the effect of host as,

1. Racial differences in pathogen affect differentially the observed LP1.
2. As seen in earlier studies (Section I), shorter LP1 in the virulent races compared to avirulent wild types was associated with decreasing rather than increasing ULD or USM.

The relatively satisfactory fit of the data for ULD and USU to the normal distribution further supports the polygenic inheritance of these traits. Possibly, the traits of disease severity are controlled by more genes compared to those traits of disease timing.

The absence of significant correlation between ULD and USU, but the presence of high correlation between ULD and USM, and between USM and USU, suggest that in some plants increased USM may be due to the higher uredinial number of those plants (cf. Chapter 7).

Transgressive segregation for quantitative resistance has been observed also in other systems (Gavinlertvatana and Wilcoxson, 1978; Skovmand et al, 1978; Wallwork and Johnson, 1984). Wallwork and Johnson (1984) further suggested that such transgressive segregants would be more useful when they are derived from crosses of parents known to demonstrate 'durable' resistance. In the present study, even the susceptible cultivar contributed to higher resistance in the progeny, probably due to additive and or recessive resistance genes. This

observation agrees with the findings by Skovmand et al (1978) in wheat for resistance to stem rust, that almost all cultivars possess slow rusting genes albeit to varying degrees.

In this study, it was not possible to estimate the number of genes or factors conditioning each resistance trait. However the progeny trend in the current cross is similar to that observed for the progeny distribution in many cereals for resistance to rust, where 2 to 12 genes conditioning traits such as latent period and area under disease progress curve has been reported (see Wilcoxson, 1981 for review).

Differences between the races in eliciting degrees of quantitative resistance (all traits) in the F1 plants indicate the usefulness of employing many races in such studies. Such differential interactions for quantitative traits of disease in the segregating progeny has also been reported in other systems (Parlevliet, 1976; Milus and Line, 1980; Wilcoxson, 1981). The demonstration of 'physiologic specialisation' in the races for quantitative resistance suggests that this type of resistance may enforce selection on the population of the pathogen for increased aggressiveness (cf. Chapters 7 and 8). However, these observations conflict with the suggestions of some authors that such quantitative or rate-reducing resistance is basically a non-specific character (e.g. Vanderplank, 1968 and 1978). The antagonistic reaction observed between the races when they were mixed which resulted in decreased aggressiveness (Chapter 8), further supports the need to employ separate pure races in studies on such resistance.

The broad-sense heritability values were extremely high for most traits and moderately high for USU. This indicates the low component of the environmental variance in the data for the traits. This would have little meaning when extrapolated to field situations, because in the present study, the physical and environmental variation had deliberately been kept to a minimum. This would contrast with the field situation where the environment is spatially and temporally heterogenous. Nevertheless, the high heritability values reflect the repeatibility of the results under similar conditions. The results agree with that of Thielges and Adams (1975), who from a field study of

several poplar clones and M. medusae, also observed high heritability of resistance.

In a comparative examination within a F1 plant of the quantitative reaction to these two races (5M and 7A) and qualitative reaction to many races studied in Chapter 13, (Appendix 8), no clear pattern of association between the two types of reactions emerged. For example, many plants which had high quantitative resistance (Long LP1, low ULD or USM) were highly susceptible (IT=4) to certain races. This conflicts the opinion of certain authors that defeated resistance genes may act as rate-reducing (quantitative) resistance (Riley, 1973; Nelson, 1979) but supports the observations of others (e.g. Ayers et al, 1981; Skovmand et al, 1978; Johnson, 1984), that these two types of resistance need not be same. There were a few plants with broad spectrum qualitative resistance and also high levels of quantitative resistance, and thus it may be possible to select individual plants which combine resistance to many races 'slow rusting' background to virulent races.

The difficulty of selection, from a large number of crosses exposed to many races, of appropriate plants with broad spectrum resistance and which are also relatively stable in their reaction to all races, could be overcome by adopting the stability analysis technique discussed by Jenns et al (1982). Also, Rees et al (1979), Thompson and Rees (1979) have discussed the pattern analysis techniques to identify certain parameters of resistance that are most important, and to group the cultivars based on their resistance traits into several classes to enhance selection. When, principal component analysis was used to analyse the F1 plants of this study, the high latent vector loadings for LP1 indicated that selection for long LP1 is likely to be accompanied by desirable concentration of the other favourable traits of resistance. Also, by cluster analysis, when the F1 plants were truncated based on their resistance response in terms of all traits, few plants with desirable traits could be selected and are being planted in the field for further testing (details of these methods and results are not presented here).

The occurrence, in this limited study of a single cross of poplar, of many genes eliciting qualitative and quantitative resistance

to various races of M. medusae is significant and similar results were observed with studies of other crosses (Appendix 9). While the transgressive segregants offer an opportunity to select plants with higher degree of resistance compared to the parents, due to the presence of additive interaction, additional improvement would be possible by futher hybridization and selection.

The appropriate deployment of such cultivars to manage the pathogen population and its evolution, so that host-pathogen system is more balanced should receive high priority. Knowledge of pathogen evolution and its interactive role with the varying host and physical environments would thus help in more meaningful management of disease via host resistance.

14.5 SUMMARY

The inheritance of five traits of quantitative resistance to two races of M. medusae was studied in a cross of P. deltoides clones. The resistance appeared to be polygenically inherited, as the F1 plants were continuously distributed for all the traits. Again, for all the traits, considerable transgressive segregation and quantitative race-specificity was observed. In most traits, based on the skewness of the distribution, higher resistance appeared to be recessive to lower resistance. The latent period was highly negatively correlated with the traits of disease severity. The apparent additive genetic control and the observed transgressive segregation, suggests a potential for rapid improvement in the level of resistance by hybridization and selection.

GENERAL DISCUSSION

CHAPTER 15

GENERAL DISCUSSION

15.1 INTRODUCTION

The leaf rusts are among the most important diseases of poplars (Steenackers, 1972; Thielges, 1984). Zobel (1982), in an analysis of various strategies to control diseases of forest crops, concluded that 'breeding for resistance to pests is absolutely mandatory'. Sometimes, disease management by host resistance, has met with failure due to the variable nature of the pathogen. Johnson et al (1967) in a treatise on cereal rusts, concluded that '...it is a tribute to the enoromous plasticity and adaptability of these pathogens, which, in one way or another, circumvented the control measures that have been devised'.

In the present study, genetic and related epidemiological aspects of the host-pathogen interaction in the Populus - M. medusae system were investigated. However, as noted elsewhere (Chapters 2 and 13), the perennial nature of the host and lack of sexual cycle in the pathogen are serious constraints to making precise genetic analysis of this sytem. While detailed information on genetic aspects is difficult to obtain within less than three year time, even a little information may be of use in understanding the strategies adopted by the pathogen and thus to tailor the management of the system to circumvent these.

This chapter examines the broader implications of the results presented in the foregoing chapters, and emphasises in particular the common or conflicting results obtained. Although, a logical development and discussion of certain ideas to provide a framework for consideration of current knowledge, has been attempted, a degree of speculation was considered justifyable. The topics investigated ranged widely, hence, for brevity, only certain significant features have been discussed here. For clarity and ease of presentation, the subject is organised

into sections covering related information.

15.2 VARIATION IN THE PATHOGEN

Future evolution of the pathogen is perhaps the greatest uncertainity in plant breeding programmes (Micke in Kwon and Oh, 1977). Stakman (1947) has described plant pathogens as 'shifty enemies'. In M. medusae the apparent genetic variability for virulence and aggressiveness on different host and in various physical environments observed in the present study, supports these opinions.

Due to the asexual nature of the pathogen population studied, many of the observed genetic changes have been inferred as due to mutations. While this is compatible with the broader definition of mutation, that is 'as any sudden heritable change', mechanisms such as mitotic recombinations, parasexuality, heterokaryosis etc. may have contributed to the observed variation. For example, Day (1972), commenting on results of Griffith and Carr (1961)'s work on Puccinia coronata of Oats (see Chapter 5), suggested that the wider virulence spectrum of the mutants induced by UV rays in this pathogen may be due to genetic recombination rather than induction of many separate but simultaneous mutations.

An allele for avirulence has been generally suggested to function as an active inducer, interacting with host resistance gene, to result in incompatibility (Rowell et al, 1963; Day 1972 and 1974; Ellingboe, 1977 and 1978; Pryor, 1977; Keen, 1981). Thus it has been assumed that loss of the avirulence allele by chromosomal deletion would lead to virulence (Flor, 1958; Chapter 6) and this hypothesis is supported by mutation frequency kinetics of M. lini by Schwinghamer (1959). However Burnett (1975) has argued, that it is improbable that isolates of Venturia inequalis (Cooke) Wint., known to be virulent on 19 resistance genes in apple (Day, 1974) would have incurred deletions at all these sites. Both the, radiation induced and natural, virulent mutants of race 4A of M. medusae in the current investigation (Chapter 5; Tables 5.1 and 5.5) exhibited similar wide virulence spectrums and

thus, comparable arguements to those of Burnett (1975) can be made for the cause of virulence in the mutants of the present study. However, the results of the reciprocal temperature-transfer study (Chapter 11) support the concept of active interaction of avirulence and resistance genes. Possibly, the overall observations could be best explained by the activity of qualitative regulatory/supressor genes, similar to those observed in M. lini (Lawrence et al, 1981b) which influence the structural a/virulence genes. Many such super-supressors, where a single locus influences the activity of many other producer loci, turning them on and off, are known in other fungi (Hawthrone and Mortimer, 1968; Burnett, 1975). In M. medusae, similar supressor/s of avirulence may be present and mutation of such allele which.results in actvity, could lead to supression of the avirulence alleles and thus lead to virulence. This hypothesis is consistent with the concept of avirulence as an active allele, and with observations in some systems, where back mutation to avirulence has been noticed (Green, 1975; Luig, 1978a). Watson and Luig (1968a) have also suggested the occurrence of supressor genes to explain the increased virulence of some races of P. graminis f. sp. tritici on wheat, and recently, the regulatory genes have been suggested to play a major role in the evolution of plant pathogens (Tepper and Anderson, 1984).

An increase in the spectrum of virulence was paralleled by a decrease in their aggressiveness in the virulent mutants on some, but not all, susceptible cultivars (Tables 6.2 and 8.2; Chapters 6 and 8). Some authors have suggested that avirulence alleles support aggressiveness of the pathogen in absence of corresponding alleles for resistance in the host (Vanderplank, 1975; Ellingboe, 1977; Gabriel et al, 1979), and thus, supression of such avirulence may lead to a decrease in aggressiveness of the pathogen (McIntosh and Watson, 1982). However, in the present investigation certain exceptions to the general patterns were noticed. For example, race 5A was virulent on cv. 7-2 but four of the five (radiation induced) virulent mutants were avirulent on this cultivar. Also, an increase in aggressiveness with an increase in the virulence spectrum on certain cultivars (Table 5.3; Chapter 5) was noticed. Thus, it is difficult to make broad generalisations on the precise nature and role of virulence or

avirulence alleles, and their relationships with aggressiveness.

In races of M. medusae, natural mutations to virulence seems to be of common occurence. Natural mutations to virulence on cv. T-173, and other cultivars were observed in both races 4A (Chapter 4) and 5A (Chapter 8). In contrast, mutation to complete avirulence on cv. T-173 or other cultivars (except on cv. 7-2; Chapter 5) was not recorded in the races even when they were cultured for several generations on cv. I-488 (Chapter 8). Although it was expected that reduced aggressiveness, associated with such unnecessary virulence, may help the frequency of avirulence alleles to increase, relatively longer generations of culture on the susceptible cultivar may be required for the race mixtures to become completely avirulent on the resistant cultivar (Leonard and Czochor, 1980).

Variation for quantitative traits in M. medusae was also observed in most studies. Such traits are believed to have a polygenic base (Day, 1974; Emara and Sidhu, 1974; Caten, 1982) but mutation of polygenes has been less understood in plant pathogens. It is reasonable to assume that in plant pathogens, much demonstrated variability for aggressiveness traits (Day, 1974; Parlevliet, 1978; Hill and Nelson, 1983), including those from this study, would have their origin in mutation. This observation is supported by the increased aggressiveness for some traits on cv. I-488 (ULD and USM; Table 7.2) in the irradiated, when compared to non-irradiated, population of the race 4B over serial culture (Chapter 7). Further assortment of the variability may be brought about by forms of asexual recombination. Such variability in aggressiveness, particularly with respect to cultivar specificity, would be an important requirement for the pathogen in its adaptation to the host and physical environment. However, the observed plateaus attained in some of the traits in race 5M when it was cultured on a cultivar T-173 for a few generations (e.g. LP1 and USM; Table 8.2; Chapter 8) may limit further evolution of the pathogen, and has been termed 'genostasis' (Bradshaw, 1972). Such limitation may have a physiological explanation (Chapter 14).

The occurrence of variability in the pathogen for environmental variables such as temperature and light intensity, is indicative of a

further dimension in the plasticity of the pathogen. Ellingboe (1978), suggested that most high-temperature-sensitive mutations are missense mutations, involving changes in the primary structure of protein which in turn affects the tertiary structure. Such an hypothesis supports the theory of active recognition by the avirulence alleles in the pathogen of the resistance allele in the host, since high temperature may lead to loss of protein function and thus result in compatibility. However, the pattern of temperature-sensitivity observed in M. medusae with clones of poplars, is frequently the reverse of those observed by Ellingboe (1978), and cited by Vanderplank (1978), i.e high temperature results in incompatibility rather than compatibility (Tables 10.1 and 11.1; Section II). Recently, many evolutionists (e.g. Hederick and McDonald, 1980; MacIntyre, 1982) have argued that major adaptive shifts probably are not the result of gradually accumulated changes in protein structure but are most likely due to changes at the level of gene regulation. These views are supported by some experimental evidence (Devonshire, 1977; McDonald et al, 1977). McDonald (1983), even suggested that environment itself influences the rate and extent of mutations necessary for adaptation, and that mutation rates may increase dramatically during periods of environmental challenge.

Thus, the populations of M. medusae, although of mono-urediniospore origin, and asexually reproducing, possess considerable potential variation for significant adaptive response to variation in host genotype, temperature and light intensity. Similar possibilities may exist in the population to adapt to other environmental features such as photoperiod, which has been shown to affect the virulence and aggressiveness of M. medusae (Singh and Heather, 1983).

15.3 RELATIONSHIP BETWEEN VIRULENCE SPECTRUM AND AGGRESSIVENESS

While a few authors have opined that the virulence spectrum and aggressiveness in the pathogen are separate factors with no interrelationship (e.g. Green, 1975; Bronson and Ellingboe, 1981), the present studies with M. medusae suggest, that these two factors may be associated. The comparative analysis, of natural or radiation induced,

virulent mutants and their respective wild types, on a range of cultivars (Chapters 5 and 8), suggested that while aggressiveness levels on a particular cultivar varied between the races (or isolates), such differences were more pronounced between races which differed in their virulence spectrum (i.e. mutants and the wild type). While on certain cultivars, an increasing aggressiveness was observed with an increase in spectrum of virulence (Chapter 5), decreasing aggressiveness was evident on cultivars such as I-488 and Italica, which are regarded as universal suscepts (Chapters 5 and 6). Further, in serial culture studies increase in aggressiveness of the races on particular cultivars was paralleled by decreasing aggressiveness on certain other cultivars, and suggesting a possible trend of decreasing virulence spectrum (Chapter 8). Consistent association of broader virulence spectrum and low aggressiveness in the races on certain cultivars could be exploited in the disease management by deploying those cultivars. Similar observations were made by Wolfe (1984) with powdery mildew of barley, and by Alexander et al (1984a) with rust of beans. These results also suggest that virulence and aggressiveness on a particular host genotype can be influenced by a non-corresponding host resistance (Wolfe et al, 1983; Alexander et al, 1984a). Wolfe (1984) has also suggested that this conclusion is somewhat contrary to the generally accepted view of the gene-for-gene theory, which assumes an all-or-nothing relationship between a host resistance gene and the corresponding gene for virulence, and no relationship between the host gene and other, non-corresponding genes for virulence. Nevertheless, it would be consistent with the possible natural evolutionary trends in the pathogen, where, on natural diverse host populations, there is a trade-off between selection for flexibility (virulence spectrum) on a wide range of hosts and of aggressiveness on specific hosts over the infectious season to suit the existing host patterns (Barrett, 1980; Wolfe et al, 1983).

The differences in aggressiveness between the virulent and avirulent races, may be responsible also for the avirulent race being a slightly better competitor on susceptible cultivars in the race mixture studies (Table 8.3; Chapter 8). Had the racemixture been serially cultured on certain cultivars on which the virulent race was more aggressive compared to the avirulent race (Chapter 5), then the former

race would possibly have been a better competitor. While this may be explained on the basis of 'weak' and 'strong' host resistance genes (sensu Vanderplank, 1968), the results of serial culture also suggest that such a classification depends on the particular cultivar on which the races were cultured. For example, the eleventh generation isolate of the virulent mutant races with increased aggressiveness on cv. I-488 had decreased aggressiveness (most traits) on cv. T-173 and vice versa for LP1 (Table 8.2).

While the present, and certain other observations (Leonard, 1969; Flor, 1971; Wolfe and Barrett, 1980; Chin and Wolfe, 1984), support the theory that the virulence spectrum (sensu the host range) and the aggressiveness on a specific cultivar are inter-related, it is difficult to propose reasons for such an association. As indicated (Chapter 6), the chanelling of avirulence alleles, which function in promoting aggressiveness on a susceptible cultivar, to function in recognition of resistance, may lead to the reduction in fitness of the race. Similarly, on those groups of cultivars where positive association between the virulence spectrum and aggressiveness exists, virulence alleles may act additively to counter all the resistance genes in the cultivar for complete expression of susceptibility (Simons and McDaniel, 1983). This theory appears more plausible than that postulated as due to linkage (Chapter 6). Linkage could be broken by recombination and other mechanisms, and selection for a wider virulence spectrum combined with increased aggressiveness on all cultivars (so called 'super races') over serial culture would then be a possibility; such a development was not observed in the present investigation (Chapter 8). Possibly, genetic limitations impose constraints on the combinations required for the production of such a race.

In a conceptual sense, the virulence spectrum of the pathogen on the host parallels that of adaptation by the pathogen to a wide range of temperature or light intensities. In the selection studies for these environmental variables (Chapter 10), those isolates with broader environmental range (temperature and light intensity) were also more aggressive. While this may have been partly an artefact of the experiment (generations of serial culture), the specificity of the low temperature and low light intensity isolates to certain environments of

incubation and the differential interaction between the isolates and environments observed (Chapter 10), suggests that it is unlikely that a single biotype with a broad spectrum adaptability to heterogenous environments (to many elements) would arise. For instance, isolates selected for virulence at high light intensities were avirulent at high temperatures and vice versa. Further, the adaptation to high temperature or high light intensity was associated with the host genotype on which the adaptation occured. Finally, despite the observed high aggressiveness of the high temperature or high light intensity isolates, the frequency of these isolates in the base population was low, suggesting that at low temperature/low light intensity, selection was operative against 'unncecessary' environmental tolerant variants.

The results of many laboratory studies dealing with virulence and aggressiveness conflicted with those of field studies (Loegering, 1951, Watson and Luig, 1968b; Martens, 1973; Falhati-Rastegar et al, 1981). The interaction of many other factors such as inoculum density, physical environmental variables etc. possibly influences the field results, hence, the trends of results reported in this thesis, should be tested under field conditions in polycyclic disease situations, employing the natural variants from the field.

15.4 RACE-CULTIVAR SPECIFICITY

Specificity is used to imply an adaptive matching of host and pathogen individuals (Scott et al, 1980). The specificity between host and pathogen genotypes is recognised on the basis of differential interaction, and four such classes of interaction, not all equally distinct, are recognised : qualitative interaction with or without reversed ranking and quantitative interaction with or without reversed ranking (Scott et al, 1980). In the present study, where the reaction of many isolates/races of M. medusae with many cultivars of poplars were assessed, all the four classes of specificity were observed, while the cultivar X race/isolate interaction in the ANOVA was almost always a significant source of variation in a parameter (Chapters 5, 6, 7, 8, 13 and 14; Appendix 8). Further, the ranking of isolates for one trait of

aggressiveness on a cultivar was somtimes inconsistent with those of other traits (e.g. Chapter 6), indicating that the extent of specificity was often dependent on the particular trait observed.

While the specificity for qualitative virulence or resistance, particularly when accompanied by a reversal in ranking (Table 5.1; Chapters 5 and Table 6.1; Chapter 6) is suggestive of the gene-for-gene basis of interaction of the host and pathogen (Flor, 1956 and 1971), the apparent specificity for quantitative traits such as latent period, uredinial number and sporulation are sometimes difficult to explain in such simple genetic terms. Parlevliet and Zadoks (1977) have suggested that such interactions result from the integrated reaction of major and minor genes in the host and pathogen on a gene-for-gene basis.

Many of the results in the present study, particularly on qualitative interactions, have been explained assuming a gene-for-gene relationship as the simplest genetic basis for such interactions. For example, the observation that the dominant gene for resistance to race 5A in cv. T-173 was overcome by the virulent mutants derived from this race, is consistent with the concept of a gene-for-gene relationship. Similarly, the differing segregation ratios in the progeny of the cross between cvs. 60/122 and T-173, atleast with certain races (Chapter 13; Appendix 8), can simply explained on the basis of a gene-for-gene interaction.

While many authors have proposed that quantitative resistance in the host and aggressiveness in the pathogen do not interact differentially and thus are 'non-specific' (e.g. Vanderplank, 1968), such views have been questioned by others (e.g. Johnson and Taylor, 1976; Parlevliet and Zadoks, 1977; Kulakarni and Chopra, 1980; McIntosh and Watson, 1982; Johnson, 1983 and 1984). The attractive feature of 'non-specific' resistance, providing this means that the resistance is expressed equally against all pathogen biotypes, is that it could be expected to be stable (Vanderplank, 1968; Person *et al*, 1983). The proposed stability would result from the absence of host biotype selection pressures on the population of pathogen biotypes. However, the apparent universal occurrence of specifcity in cultivars of poplars to races of *M. medusae* (Sections I and III; Singh and Heather, 1982b and

1983) and of M. larici-populina (Heather and Chandrashekar, 1982), raises doubts on the occurrence of such 'non-specific' interactions, atleast in this system. Many workers, however, are optimistic about identifying such 'non-specific' resistance. In their model, Person et al (1983) have shown that when polygenes of hosts and pathogens are assumed to interact non specifically (additively), stability could be achieved. Attainment of such stability via 'non-specific resistance' is being pursued in many pathosystems (e.g. Beek, 1983; Robinson, 1983). Because of constraints in identifying such apparent race non-specific resistance, and conflicts about its role, Ellingboe (1975) has suggested that race-non-specific resistance is one which has not yet been shown to be race-specific. Wolfe and Schwarzbach (1978) have questioned the existence of race-non-specific resistance since 'it precludes the possibility of pathogen evolution'. In the analysis of field observations, the race X cultivar interaction component in the ANOVA may be confounded with the error component (Parlevliet, 1981), and thus race-specificity may go undetected. In practice, as pointed out by Caten (1974), it is not simply the presence or absence of a differential interaction that matters but their magnitude relative to the differences in resistance between the cultivars, i.e. their capacity to excercise selection pressure. Thus, with the adoption of a suitable statistical approach, it may be possible to identify those cultivars which are less interactive with the variations in the pathogen (Hamid et al, 1982; Jenns et al, 1982).

15.5 COMPATIBILITY AND INCOMPATIBILITY PHENOMENA

Although it is difficult to define precisely, compatibility implies an association of pathogen and host species resulting in susceptibility of the latter and reproduction of the former, while incompatibility is a resistance reaction within the framework of this association, i.e. the ignoring the non-host resistance (cf. Ellingboe, 1981; Heath, 1981). Rowell et al (1963), interpreting the quadratic check of host and pathogen genotypes, suggested that resistance alleles in the host and avirulence alleles in the pathogen interact to elicit an incompatible reaction. Subsequently, Browning (1974) suggested that, during

evolutionary time, such major gene systems have become superimposed over the basic compatibility system. The genetic, evolutionary, physiological and biochemical implications of both systems have been discussed (Ellingboe, 1977; Bushnell and Rowell, 1981; Heath, 1981; Keen, 1981; Parlevliet, 1983a). While some authors have suggested that specific and active recognition is for incompatibility or resistance (e.g. Ellingboe, 1977), others have suggested that it is for a compatible or susceptible reaction (Favret, 1971; Ward and Stoessl, 1976; Hiura, 1978; Vanderplank 1978 and 1982). The objectives of the present study did not include a specific analysis of these viewpoints, but the results permit discussion and speculation on relevance to the current concepts.

The incompatibility system (Model I) has been proposed to act on a gene-for-gene basis, with the recognition for incompatibility (-) between the alleles for resistance and avirulence (Ellingboe, 1977; Parlevliet, 1983a). Usually, but not necessarily, both alleles are dominant, and the absence of one of the alleles, by default, leads to a susceptible (+) reaction (Table 15.1) (Ellingobe, 1978; Parlevliet, 1983a).

Table 15.1 The incompatibility system (Model I) (Parlevliet, 1983a).

	Host allele	Pathogen alleles	
		Avirulence	virulence
		A_	aa
Resistance	R_	-	+
Susceptibility	rr	+	+

In the present study, the reaction of cvs. I-488, T-173 (Table 6.1; Chapter 6) and a few other cultivars (Chapter 5 and 8), to both the wild type and virulent races, are consistent with this hypothesis. For example, the genes for resistance in cv. T-173 to races 5A and 4B appear

to be dominant, while the ease with which the virulent, is obtained from the avirulent, race suggests that avirulence may be dominant (Chapter 4). The studies with temperature activation of resistance, also support the theory that active recognition is for an incompatible rather than a compatible reaction, and that the former is potentially epistatic to the compatible reaction.

However, the results of the reaction of virulent mutants with certain other cultivars are inconsistent with this theory. For example, the mesothetic (necrotic flecks and few uredinia) reaction of cv. W-79/307 to the wild type race 5A was changed to complete susceptibility with the virulent mutants (Table 5.1; Chapter 5). If the necrotic flecks, elicited by the avirulent race, are the results of interaction with the corresponding resistance gene in this cultivar, it is difficult to explain the absence of epistasis of this incompatible reaction. Further, the increased virulence spectrum of the irradiated mutant (5M series) resulted in an increase in aggressiveness on this cultivar (Table 5.3). Thus, this cultivar (W-79/307) may carry many genes for resistance and absence of recogniton of some these genes by the wild type (race 5A) may not result in complete susceptiblity. The virulent mutants, because of their increased virulence spectrum, could negate all these genes to achieve complete susceptibility. However, in this cultivar (W-79/307), the temperature sensitive resistance reaction to race 4C was epistatic to the potential susceptible reaction of race 4M, when the latter was inoculated four days after the inoculation with the former race (Table 11.3; Chapter 11). Further, on this cultivar, the natural virulent mutants did not exhibit increased aggressiveness in contrast to the radiation induced mutants (Table 5.6). Possibly, as suggested by Kuc (1983), the resistance reaction depends not merely on the presence or absence of genetic information but whether this information is expressed soon enough and with sufficient magnitude. Watson and Luig (1968a) suggested that such intermediate reactions may result from incomplete virulence or heterozygosity. Eskes (1983a) has discussed certain other factors responsible for such reactions.

The present theories are inadequate to explain the loss of virulence in the induced virulent mutants of race 5A to cv. 7-2 (Table

5.1; Chapter 5). This change suggests that virulence of the wild type race 5A towards this cultivar may not be due to the deletion of avirulence alleles, for it would then be impossible for the avirulence allele to arise at this locus by mutation. Thus, the supressor/regulatory gene hypothesis (Chapter 5) would best explain the observed reactions as it does not assume the deletion of an avirulence allele to result in virulence. However, such interactions would not correspond to the gene-for gene theory sensu stricto, because of the lack of numerical equivalence between the genes in the host and the pathogen.

The present investigations suggest that variations in basic compatibility exists at differing degrees in both host (susceptiblity) and pathogen (aggressiveness) and that this is predominantly interactive. According to Parlevliet (1983a), the basic compatibility system (Table 15.2; Model II) operates on a reverse gene-for-gene basis, where the recognition is for compatibility, operating in polygenic fashion between genes for resistance/susceptibility in the host and aggressiveness in the pathogen (Table 15.2) (Parlevliet and Zadoks, 1977) :

Table 15.2 The basic compatibility system (Model II).
(modified from Parlevliet, 1983a).

Host Resistance		Pathogen aggressiveness	
		High	Low
		A-	aa
Low	S-	+	+
High	ss	+	-

Although presented as a single locus, this model represents a multilocus system. The low resistance (high susceptibility) is dominant over high resistance (low susceptibility) in the host and high aggressiveness is dominant over low aggressiveness in the pathogen. Since these factors are measured by quantitative parameters, the extremes would result in either higher susceptibility or resistance, and

thus the disinction between + and -, is one of a degree rather than kind. The quantitative components of resistance in the cross cvs. 60/122 X T-173 (Fig. 14.1; Chapter 14) and in other crosses (Appendix 9) appear to be inherited polygenically, and higher resistance for most traits was recessive to lower resistance . Similar results have been obtained with many such quantitative studies on resistance in other systems (e.g. Parlevliet, 1977; Kulakarni and Chopra, 1980; Wilcoxson, 1981; Eskes, 1983b). Also, the higher aggressiveness in the pathogen has been observed to be dominant to lower aggressiveness in Ustilago hordei (Emara and Sidhu, 1974). The decrease in aggressiveness of the wild type race 5A of M. medusae upon mutation (Chapter 6) is consistent with this suggestion, as mutations are usually from dominant to recessive (Parlevliet, 1983a). Thus, as suggested by Ellingboe (1976) and Parlvliet (1983a), in a basic compatibility model, [illegible] recognition seems to be for susceptibility.

In the evolutionary context, the onus is on the pathogen to evolve gene products to result in the compatibility system (Parlevliet, 1983a), while in the incompatiblity system, it is on the host to recognise the product of avirulence in the pathogen (Heath, 1981). In addition, Ellingboe (1978), has suggested that during evolution, the pathosystem evolves from a compatibility system (model II) to incompatibility system (model I), and thus in new pathosystems such as Cochliobolus victoriae (Drechs.) Nelson on oats, the available evidence is suggestive of only compatibility system (model II), and with more evolved biotrophic pathogens, the evidence is for the incompatibility system.

In the Poplar-M. medusae complex, there is circumstantial evidence for occurrence of both systems (Tables 15.1 and 15.2), and thus the identification of the particular model depends on the method of analysis (qualitative or quantitative) adopted. If we assume that both systems are integrated and interactive, much of the variation observed in either system can possibly be understood. This assumption would account also for the complexity of the system, wherein the relative influence of each system in the ultimate disease expression would be expected to vary depending on the particular genotypes of the host and pathogen, in interaction with the physical environment. These interactive relationships between the components of the incompatibility

system (virulence and resistance alleles), the compatibility system (susceptiblity and aggressiveness factors), and the physical environment would influence the disease expression and hence the evolution of the pathosystem.

15.6 EVOLUTION OF THE PATHOGEN AND STABILITY OF THE SYSTEM

Breeding plants for resistance to pathogens is breeding against a flexible adversary (Heybroek et al, 1982) and the present study with M. medusae on poplar further emphasises this point. Evolution is a continuous process and in natural pathosystems the association of the host and pathogen can have four possible consequences (Barrett, 1984) :

1. Chance fluctations in population numbers eliminate the host, the pathogen or both.
2. The pathogen eliminates the host, and if the pathogen is a biotroph, it also is eliminated.
3. The association persists but the host eventually evolves complete resistance/immunity and the pathogen becomes extinct or ceases to be a pathogen on the particular host.
4. An equilibrium between host and the pathogen is attained and the association persists.

Only the last of these outcomes will leave any evidence of the association ever having existed, and much of our current observations and interpretations are based on this association (Barrett, 1984).

Evolution concerns variation and selection in their interplay with one another (Mather, 1973). According to Harper (1977), the only way to study such a dynamic process is to perturb the system. Whilst there may be moral or ethical problems in disturbing the natural ecosystems in order to explore the underlying processes (Barrett, 1984), these problems are avoided in laboratory studies, such as those reported in the thesis.

The objectives of the pathogen, particularly a biotroph, in evolution, however, are conflicting. It must be fit to survive and pass on the maximum number of progeny (in the Darwinian sense), but must ensure also that the host survives. Such harmony between host and pathogen may exist in natural pathosystems and may result from a number of interactive factors (Haldane, 1949, Futuyma, 1979; Leonard and Czochor, 1980). In some systems, where man has a predominant role, this harmony may be disturbed and epidemic diseases result (Watson, 1970; Marshall, 1977; Nelson, 1979). An understanding of the evolution of the pathogen may help to restore stability to such a system by reciprocal structuring of the host population. An analysis of microevolutionary processes, as evidenced in this investigation, may yield clues on the naturally occurring micro and macroevolutionary patterns (Gould, 1977). Due to the short generation time and high fecundity of some pathogens, rapid shifts in population, in response to biological and physical selection pressures, could occur in a relatively short time span, e.g. Helminthosporium blight of corn, Endothia blight of chestnut, Ceratocystis (Dutch elm) disease of elms (Day, 1974) illustrate this suggestion. The pathogens may act as strong selective forces on the hosts and in natural populations, hosts may develop polymorphism over time (cf. Haldane, 1949; Harlan, 1976). Pathogen induced changes in the host populations can also occur over a relativley short time span (e.g. studies with biological control of skeleton weed in Australia; Burdon et al, 1981).

Directional selection for virulence and aggressiveness in M. medusae is exerted by host genotypes (Chapters 7 and 8) and by environmental variables (temperature and light intensity) (Chapter 10). Further, the wide changes in the virulence spectrum on a range of host cultivars observed in both natural and irradiated populations (Chapter 5), suggests that this pathogen has significant potential flexibility for adaptation to a considerable host range. While such directional selection at the extremities of the scale (Chapters 7, 8 and 10), could obviously result a consistent drift towards higher levels of disease, a disruptive selection can bring about a multimodal response. For example, the studies with temperature and light intensity (Chapter 10), demonstrated a degreee of adaptation in virulence and

aggressiveness in the pathogen to the specific regimes of incubation. In nature, due to the cyclic form of certain environmental features, polymorphism in the pathogen may still result, since individual isolates specific to a particular seasonal niche are replaced by others when the environmental changes occur. Environmental heterogeneity over space can also cause polymorphism. Further, Sharma and Heather (1982) have suggested that, the observed interaction of hyperparasites like Cladosporium spp. with the leaf rust in poplar, may provide further homoeostatic selection pressure on the pathogen. Such polymorhphisms due to spatial and temporal heterogeneity of the biological and physical environment may contribute to the stability of the system (Ludwig, 1950; Merrrel, 1981).

Similarly, disruptive selection by host genotypes can be enforced by deploying cultivar mixtures which do not support directional selection for aggressiveness in the pathogen on all host genotypes. Significant disassociation of the aggressiveness trends have been observed in the virulent races on certain cultivars (Chapter 8). This can be exploited in poplar plantings to keep the disease levels to a minimum and to prevent domination by a race with wide virulence spectrum combined with high aggressiveness on all the cultivars, because of the evolutionary dilemma faced by the pathogen in such situations. Such a system would exploit the variability in the pathogen and race-specificity in the host, the two most cited constraints in breeding for disease resistance (Day, 1974), to achieve disease control.

In other systems, several means have been proposed to achieve stability and minimise losses due to disease (Chapter 12). The gene pyramid approach (Nelson, 1979), the safety of which is based mainly on the genetic probabilities (Person et al, 1976), may not be a suitable approach in poplar leaf rust sytem, because, 1) the pathogen has been observed to mutate with relative ease to very wide virulence spectrum (Chapters 4 and 5), 2) The 'defeated' resistance genes do not necessarily retard the aggressiveness of the pathogen (Chapters 5 and 14). 3) The homogeneity of the deployed host population is implicit in this approach, and thus, if a pathogen population with wide virulence spectrum should arise, it may develop also high aggressiveness, because

there is no scope for selection against unnecessary virulence and/or aggressivenes on other cultivars. Further, as observed on some cultivars (Chapter 5), the pathogen can increase in virulence to match additional resistance genes to result in complete susceptibility. Thus numerous major resistance genes within a cultivar may possibly be a liability for the host rather than an asset, and further research is needed in this area.

The gene pyramid approach would involve extensive hybridization and backcrossing; these are difficult and time consuming in tree crops. A multiline system may also be unsuitable for these reasons. The latter system also has the risk factor of a uniform background genotype of the host, which may enforce directional selection pressure on the aggressiveness in the pathogen (Leonard, 1969a; Wolfe, 1984).

Due to the apparent exclusive occurrence of race-specificity in the cultivars and F1 plants (Section I and Section III) for both qualitative and quantitative traits of resistance in this system, the search for apparent non-specific resistance is not justified. Indeed, the validity of the proposing the existence of such a resistance, particularly to a biotroph like M. medusae, appears doubtful because of the apparent dynamism in the evolution of the pathogen which is evident from serial culture studies (Chapter 7, 8 and 12).

Cultivar mixtures have been suggested as a strategy to manage diseases in crops since long time (Tozett, 1767), including poplars (Hartley, 1939). Many recent studies in other systems have outlined the importance of such an approach (Browning and Frey, 1969; Leonard, 1969; Heather and Chandrashekar, 1982; Wolfe et al, 1983). Further, the monoculture of cv. I-214 which comprised about 65% of the entire poplar culture in Italy, resulted in serious epidemic of Marsonnia brunea (Ell. and Ev.) P. Magn. (Thielges, 1984), suggesting the need for alternative strategies. Although the present results are based on studies conducted in an artificial experimental environment, they suggest refinements of the cultivar mixture system proposed by Heather and Chandrashekar (1982).

A large number of cultivars with broad and diverse backgrounds should be obtained initially from a germplasm collection, hybridization and or selection. In a field nursery established from these cultivars, initial selection for silvie characters such as straightness of the stem, small branch diameter, flat branch angles, rapid growth rate, and wood characters such as basic density, fibre length could be conducted. From this collection, certain cultivars towards which virulence does not arise readily, or even if it occurs, the particular pathogen biotype lacks competitive fitness in the absence of a specific cultivar(s), should be selected (e.g. cvs 40-2 and 10-3; Chapter 5 and 8). Further, by serial culture studies, groups of cultivars which favour disassociation of virulence and aggressiveness could be recognised (e.g. cvs. 7-2 and 7-4 in Chapter 5; cvs. I-488 and T-173 in Chapter 8). Ideally, such cultivars should carry major resistance genes embedded in a 'slow-rusting' background. Obviously, amongst many such cultivars, with varying degrees of quantitative resistance (for numerous traits), with differing specificity to many races, it may be difficult logistically to select a few 'elite' cultivars suitable for deployment. Pattern analysis, such as those suggested by Rees et al (1979) and Thompson and Rees (1979), would be helpful in the selection of such cultivars within the germplasm and segregating population. If for management and silvicultural reasons, monoclonal plantings are desired for deployment on limited areas, then those cultivars which do not support directional selection of the fitness in the pathogen (e.g. 40-2 and 10-3) may be suitable. Similary, the stability analysis approach suggested by Jenns et al (1982) may enable selection of cultivars which are least interactive with the changes in pathogen genotypes.

Deployment of mixtures, or mosaics, must also consider ecological, management, quality, uniformity, cost-benefit and operational factors. For example, further epidemiological factors (e.g. urediniospore dispersion) need to be considered to determine the number of clones in the mixture or the size of monoclonal group in the mosaic planting. Further, it may be necessary to test the cultivar mixtures at different geographic locations. Monitoring of pathogen populations at random selected sites, to detect changes in frequencies and

aggressiveness on individual cultivars may be necessary in the initial stages. While Darwinian selection may favour individuals with a high degree of aggressiveness on each host, group selection may operate to reduce the flexibility of the races, in the mixed clonal plantings (Parlevliet, 1983a). If the host populations consist of mixture of cultivars which support disruptive selection and ensure negative association of virulence spectrum and aggressiveness, then the pathogen population could be expected to remain at moderate levels. Also, due to the observed antagonism between the virulent and avirulent races in mixtures on susceptible cultivar (Chapter 8), coupled with the phenomena of induced resistance on resistant cultivars (Chapter 11), polymorphism in the host, which brings about polymorphism in the pathogen, should result in lower levels of disease in heterogeneous host populations. Such complex interactions of the host, pathogen and the environment may confer stability of resistance to the system.

15.7 CONCLUSIONS

The results obtained in the present investigations are presented concisely at the end of each chapter and in the Abstract at the beginning of the thesis. The major experimental results summarised below, broadly fulfilled the major objectives of the present investigations (section 1.8; Chapter 1).

1. In M. medusae, natural mutation to virulence towards P. deltoides cv. T-173 and certain other cultivars is frequent, and could also be induced by gamma irradiation. Such a phenomenon is typical of major gene-for-gene relationship frequently observed between hosts and plant pathogens.

2. The virulent mutants exhibit a wider virulence spectra compared to the wild types on a range of host cultivars, although on a certain cultivar, (P. deltoides cv. 7-2), they exhibit loss of virulence. These changes could be best explained by the activity of regulatory genes. The trend of such changes in irradiated and natural mutants were largely similar.

3. On the cultivars studied, the increased virulence spectrum was usually associated with changes in aggressiveness in the pathogen, and on certain cultivars, particularly 'universal suscepts', the association was negative.

4. With serial culture, some cultivars exert positive selection pressure on the pathogen towards increased aggressiveness over generations of serial culture. Such increased aggressiveness, for some traits of disease severity, was higher on a cultivar when the base population of the pathogen was irradiated, and thus the variability for aggressiveness in the pathogen may have a mutational basis.

5. When virulent races were cultured separately on a susceptible (P. x euramericana cv. I-488) and a resistant cultivar (P. deltoides cv. T-173) for eleven generations, although both cultivars exerted some positive selection on the races towards increased aggressiveness for some traits on their cultivar of serial culture, a negative selection or decreasing aggressiveness for some traits on 'other' cultivar was evident. Such disassociation of aggressiveness trends in races on some cultivars may have potential in disease control by using mixtures or mosaics of cultivars.

6. In a particular mixture of races, the avirulent race was observed to be a slightly better competitor on a susceptible cultivar than the virulent race. Possibly, for a virulent mutant to be epidemiologically successful in the absence of the corresponding resistant host, it should arise in a super-aggressive biotype, with marginally higher aggressiveness than the mean of the avirulent population. However, an antagonistic interaction between the two races occurred in the race mixture on susceptible cultivar. This emphasises the desirability of promoting polymorphism in the pathogen populations.

7. Increasing temperature and light intensities of incubation over discrete generations of serial culture on a cultivar, exerted positive selection pressure on the pathogen, wherein isolates suited to specific environmental regimes are selected. In nature, because of heterogeneity of the physical environment over time and space, such plasticity and specificity in the pathogen may lead to polymorphism in the form of environmental races.

8. Using a temperature-sensitive race of the pathogen and reciprocal transfer of inoculated leaf disks across pemissive and non-permissive temperatures, recognition of incompatibility appeared to be an active and more rapidly induced phenomenon which occurred within 15 h of inoculation, while compatibility required c. four days. Further, such incompatability was epistatic to potential compatibility to a temperature-non-sensitive race, and hence such induced resistance may have an epidemiological role.

9. Simple inheritance of qualitative resistance was observed in the F1 progeny of a cross of P. deltoides cultivars, when tested for reaction to five races of the pathogen. Resistance appeared to be inherited as dominant (3 races), recessive (1 race) or additive/codominant (1 race), and possibly controlled by a single gene or two genes acting in a complementary manner depending on the race employed. Race specificity was apparent in the progeny, and the overall results are consistent with the gene-for-gene relationship between the host and pathogen.

10. When two races each virulent on both parents in a cross were employed, the quantitative components of resistance (five traits) in the progeny (those referred to in 9 above), were largely race-specific and polygenically inherited. However, numerous transgressive segregants were observed indicating the scope for selection of higher resistance. In most traits, the higher resistance was usually recessive to lower resistance, although presence of additive interaction was evident. The

high negative correlation between latent period and other traits of disease severity observed in the progeny, may permit efficient selection of slow rusting cultivars.

15.8 SUGGESTED AREAS FOR FUTURE RESEARCH

In the discussion of some the foregoing studies, certain areas suitable for further investigation have been mentioned. The desirability for further studies in the the breeding and deployment of cultivars was discussed in earlier sections of this chapter. The following aspects may be suitable also for further research on genetic and other related aspects of this pathosystem.

1. ISOLATION OF GENE PRODUCTS CONCERNED WITH AVIRULENCE IN THE PATHOGEN

The isolines of the pathogen differing in virulence, obtained by mutational approach, may be suitable also for comparative analysis for enzyme and protein differences, for possible isolation and understanding of gene products involved in avirulence (or virulence). Preliminary work has shown that large differences in isoenzymes exist between virulent mutants and avirulent wild types of M. medusae (Appendix 6). Further work may provide useful information in this area.

2. ANALYSIS OF RESISTANCE MECHANISIMS IN THE HOST

The cv. W-79/307 and race 4C (Chapter 11), becuase of the observed temperature sensitivity of incompatibility, is an useful system for studies on physiological and biochemical aspects of resistance mechanisms, as compatibility and incompatibility can be observed in the same system by environmental manipulation, i.e. without affecting the genotypes of the organisms.

3. INDUCED MUTATION TO AVIRULENCE

The leaf replica technique (Appendix 3) is suitable for isolation of avirulent biotypes. The radiation induced and natural virulent mutants could be subjected to further mutagenesis and the occurrence of possible reverse-mutation in these races towards avirulence on cv. T-173, could be tested. Isolation of such reverse-mutants would be suitable for further comparative studies, with the wild type and virulent mutants, for virulence spectrum and aggressiveness and for protein composition. Additionally this would enable further examination of the association of the virulence spectrum and aggressiveness (Chapter 6) and of possible linkage of some virulence loci (Chapter 5). A limited study in these lines was attempted by the author, but reverse mutation for avirulence in race 5M2 was not induced, possibly because of the smaller number of uredinia tested.

4. HOST SELECTION PRESSURE ON AGGRESSIVENESS IN THE FIELD SITUATION

At intervals during a polycyclic epidemic, isolates of M. medusae could be collected from various geographically isolated clones of poplar and their aggressiveness assessed on cultivars in the laboratory. This study may indicate the possibile occurrence of specificity of the isolates towards their 'own' cultivars. If such adaptation does occur in the field, it would be expected that isolates collected at the end, rather than the beginning, of the epidemic would be relatively more aggressive on the respective cultivars.

5. ENVIRONMENTAL POLYMORPHISM IN M. MEDUSAE

Isolates of M. medusae could be collected from different discrete environmental habitats in the poplar growing regions of Australia, at different times during the infectious season, and compared for their temperature and light intensity preferences in terms of aggressiveness on their respective cultivars. Such a study may detect the environmental specificity of the isolates and polymorphism for such traits.

6. IMPACT OF CULTIVAR MIXTURES ON THE VIRULENCE SPECTRUM AND AGGRESSIVENESS OF THE PATHOGEN

Different mixtures of cultivars (Chapter 15), consisting of cultivars supporting (a) positive or, (b) negative association of aggressiveness trends in the pathogen could be planted in the field nursery, and the pathogen could be periodically isolated from these mixtures. Testing of such isolates for virulence spectrum and aggressiveness on a standard group of cultivars, would provide information on, 1) the possible operations of unilateral selection for aggressiveness trends in the former group of cultivars (group 'a'), 2) Do those cultivars (group 'b'), which together favour disassociation of aggressiveness trends in the laboratory studies, support such trends in the field? Further, monitoring disease levels in the field may provide information on the stability disease resistance in two groups of cultivar mixtures over time.

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If I were to await perfection
I would never finish this book

– An old chinese proverb

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APPENDICES

APPENDIX 1

GLOSSARY OF SOME TERMS USED IN THE THESIS

The following glossary is not intended to define the terms but provides the context in which these terms have been used in the thesis, along with a brief description of them. This was considered necessary because of the conflict in the usage of certain terms in the literature. Here, isolate and cultivar refer to the pathogen and host, respectively.

AGGRESSIVENESS - The quantitative measure of certain traits of disease timing and severity induced by an isolate on a particular cultivar(s), and does not imply absence of specificity.

AVIRULENCE - The inability of an isolate to infect and reproduce on a specific host cultivar.

COMPATIBILITY - The association of an isolate and a cultivar which results in a susceptible disease reaction.

COMPETITIVE ABILITY - The capacity of an isolate to survive and maintain or increase its proportion in a heterogeneous mixture with other isolates on compatible cultivar(s).

DIFFERENTIAL CULTIVARS - A group of cultivars on which the isolates exhibit differing virulence reactions, and thus can be recognised as distinct races.

DIRECTIONAL SELECTION - Selection operative at one end of the scale which would result ultimately in changes in the isolate composition of a population towards the extreme mean.

DISRUPTIVE SELECTION - Operates in diverging ways, and thus no single isolate phenotype would be the fittest to survive, mainly due to the heterogeneity of the biological and physical environment; usually results in polymorphism where many phenotypes would be fit to exist.

INCOMPATIBILITY - The interaction of an avirulent isolate and a resistant cultivar, within the framework of compatible host-pathogen species, resulting in the lack of visible symptoms of, or less pronounced, disease reaction.

MONOCYCLE - The period (days) from inoculation of the urediniospores of an isolate on a compatible cultivar, until the cessation of the resulting sporulation. Due to the difficulty of precise assessment of cessation of sporulation, the monocycle was considered complete when at least 80% of the uredinia appear fluffy and start to disintegrate. Usually, by this time the disease progress curve in terms of ULD and USM would have reached a plateau.

QUALITATIVE RESISTANCE - A discrete and complete resistant reaction (e.g. hypersensitivity, necrosis etc.) of the cultivar in contrast to quantitative resistance.

QUANTITATIVE RESISTANCE - When the cultivar and isolate are compatible, the disease intensity measured in terms of latent period, uredinial number, sporulation, disease progress etc. is low relative to that occurring in other cultivar/isolate complexes.

RADIOSENSITIVITY - The measure of susceptibility of an organism to increasing levels of ionised radiation.

REGULATORY GENES - Those genes that influence the timing, and/or extent of activity, of other structural or producer genes. Supressor or inhibitory genes are considered regulatory genes, but affect the nature, rather than degree, of expression.

RESISTANCE - The ability of a host cultivar to prevent, or reduce, the level of the disease development and reproduction of an isolate.

SLOW-RUSTING - Results in higher, quantitative type resistance in a cultivar; especially characterised by a longer latent period with a consequent increase in the length of a monocycle.

SPECIFICITY - Qualitative race specificity occurs when certain races of the pathogen are virulent on some, but not on other cultivars. Quantitative specificity occurs when the relative level of disease induced by virulent isolates varies with the cultivar employed. Environmental specificity occurs when, within the cardinal limits on a specific cultivar, some isolates are more aggressive in certain environments of incubation than at others.

VIRULENCE - The ability of an isolate to successfully infect and reproduce on a specific cultivar.

VIRULENCE SPECTRUM - The number of cultivars, within a set of differentials, on which an isolate is virulent. This may be broad (numerous cultivars) or narrow (few cultivars).

WILD TYPE - The phenotype usually found in nature and thus isolated from the field; contrasts with the mutant type which is derived in the laboratory from the wild type.

APPENDIX 2

THE GENUS POPULUS AND HISTORY OF CULTIVARS USED IN THE STUDY

The genus Populus is divided into five sections viz.,

Section	Common Name	Important Species
Leuce	White poplar	alba, tremula, tremuloides, grandidenta
Aigeiros	Black poplar Cottonwood	nigra, deltoides, x euramericana
Tacamahaca	Balsam poplar	trichocarpa, yunnanensis simonii
Leucoides	Swamp cottonwood	heterophylla, ciliata
Turanga	-	euphratica

Species within a section interbreed, and those between certain sections (e.g. Aigeiros and Tacamahaca) are compatible also (Fig. A2.1). The species between those sections that are normally incompatible (e.g. Aigerios and Leuce; Fig. A2.1), can be rendered compatible by irradiated or mentor pollen technique (Knox et al, 1972; Pryor and Willing, 1982).

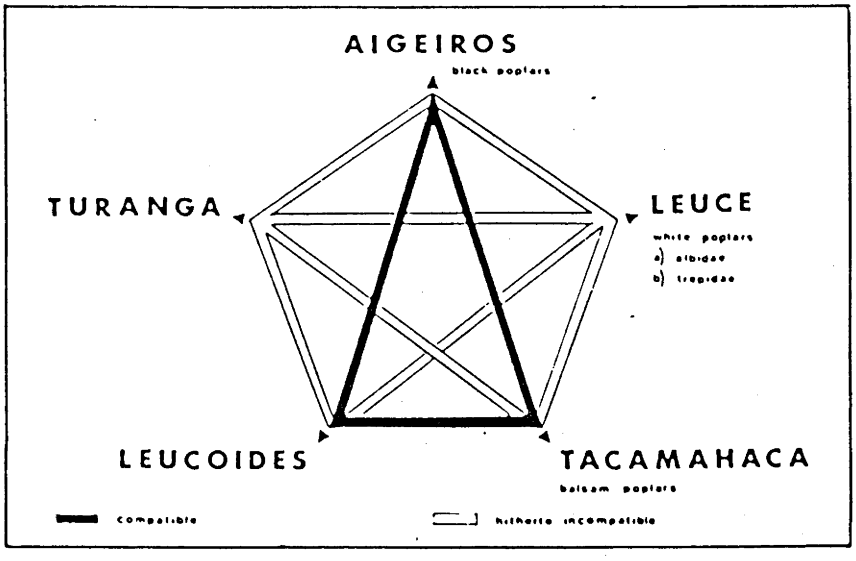


Fig. A2.1 The five sections of genus Populus, and their compatibility (Reproduced from Pryor and Willing, 1982).

The hybrid poplars derived from *P. deltoides* and *P. nigra*, are known collectively as *P.* x *euramericana*. In the following list of cultivars (Table A2.1), the first two numbers of certain cultivars (e.g. 60 and 79) refer to the year of selection or origin. The cvs 7-2 and 7-4 are half-sibs and cvs W-79/304, W-79/306 and W-79/307 are full-sibs.

Cultivar/s	Type	Parentage or Provenance	Sex	Remarks
P. deltoides				
T-173	selected seedling	College Station Texas	Male	selected in Tumut, NSW
10-3	selected seedling	Barton Rouge, Louisiana	Female	selected in Kempsey, NSW
40-2	selected seedling	Rosedale, Mississippi	Male	selected in Kempsey, NSW
7-2 & 7-4	selected seedling	Barton Rouge Louisiana	Female	selected in Kempsey, NSW
W-79/304,			unknown	
W-79/306 &	Manipulated	cvs 61/58	unknown	
W-79/307	hybrids	X T-173	unknown	
60/103	selected seedling	Texas	Male	
60/122	selected seedling	Texas	Female	
60/160	selected seedling	Stoneville Mississippi	Female	Drought resistant
60/164	selected seedling	Stoneville, MS	Female	
60/166	selected seedling	Stoneville, MS	Female	
82-PH-1	Manipulated hybrid	cvs. 60/122 X T-173	unknown	selected in A.C.T.
P. x *euramericana*				
I-154	Manipulated hybrid	*P. nigra* X *P. deltoides*	?	Selected in Italy
I-214	Spontaneous hybrid	*P. nigra* X *P. deltoides*	Female	Selected in Italy
I-488	Manipulated hybrid	*P. nigra* X *P. deltoides*	?	Selected in Italy
65/70	Manipulated hybrid	cv. 60/164 X *P. nigra* cv. Chile	Female	Selected in A.C.T.
P. alba				
Hickeliana Evergreen	Introduced clone	North Africa	Male	Semi-
P. nigra				
Italica	Introduced clone	Italy	Male	Lombardy poplar
P. simonii	Introduced clone	China	Male	

APPENDIX 3

A LEAF REPLICA TECHNIQUE TO ISOLATE AVIRULENT BIOTYPES IN LEAF RUSTS

In studies of host/fungal-pathogen interactions, avirulent biotypes are of value in identification of resistance genes in the host (Ellingboe, 1978), in investigations on pathogenesis and incompatibility phenomena (Day, 1974), in the production of quadratic checks (Rowell *et al*, 1963), in epidemiology (Leonard, 1977) and in cross protection using induced resistance (Johnson & Allen, 1975). While virulent mutants are readily isolated from rust populations, avirulent biotypes have not been selectively isolated due to a lack of suitable techniques (Day, 1974).

Race 4A, a mono-urediniospore derived culture of *M. medusae*, though initially avirulent on *P. deltoides* cv. T-173, later developed spontaneous mutants virulent on this cultivar (Chapter 4). Thus a technique, a modification of the replica plating procedure of Lederberg & Lederberg (1952), was developed to indirectly select from a mixture, biotypes of race 4A virulent and avirulent on cv. T-173. Avirulence here refers to the incompatibility of a biotype of a pathogen on a specific host cultivar. The technique was used also to isolate, from within a single race, temperature sensitive biotypes of this pathogen.

The culture of race 4A, comprising a mixture of biotypes avirulent and virulent on cv. T-173, was multiplied on detached leaves of *P. x euramericana* cv. I-488, a universal suscept, to produce fresh urediniospores which were subsequently dried over silica gel (24 h) and P_2O_5 (24 h). Leaf disks (1.7 cm^2) were punched from surface sterilised leaves of cv. I-488, grown in a rust-free glass house ($20\pm3^{\circ}$ C, 16 h photoperiod), and those containing major veins were eliminated. The freshly dried spores (1-2 mg) were deposited on 50 leaf disks and 10 coverglasses in a spore settling tower (section 2.4.2; Chapter 2). Drying of the echinulate spores reduces clumping while shielding the

leaf disks and coverglasses with the sliding lid of the tower (Figure 2.1), for the initial 10 sec after the release of spores, allows deposition of any clumps on this cover and ensures that only single spores fall on the disks. This was confirmed by examination of the coverglasses.

Inoculated leaf disks were placed on plastic foam, soaked in gibberellic acid solution (10 mg/L), located in glass Petri dishes and incubated in growth chambers at 'standard environment'. Due to the low level of inoculum used, only 5-10 uredinia developed on each leaf disk. Twenty five disks, each bearing 4-5 uredinia which were well separated from each other, were selected after 13 days of incubation [Generation I (Gen I)] when the uredinia were compact but commencing to release the spores.

Freshly punched leaf disks of the resistant cultivar (T-173) were evenly and gently pressed on each Gen I disk of cv. I-488, the leaf disk pair being held between two rubber stoppers, thus obtaining a light imprint of the spores from the uredinia. These imprinted disks (Gen II) were numbered 1-25 and located in a regular pattern on plastic foam soaked in gibberellic acid solution in a Petri dish with the polarity of each disk marked by snipping the top edge (Fig. A3.1). A similar replica inoculation was made from the disks of cv. I-488 (Gen I) on to fresh leaf disks of cv. I-488 (Gen II) which were also located in a regular pattern in a Petri dish. The Petri dishes bearing I-488 disks (Gen I) were stored at 0° while the Gen II disks were incubated as described previously. The Gen II disks of I-488 permit a check of the viability of the spores of the Gen I uredinia and allow isolation of the biotypes virulent or avirulent on cv. T-173.

Typical disks of cv. I-488 (Gen I) and of cvs T-173 and I-488 (Gen II), following the development of uredinia on the latter, are shown in Fig. A3.1. Every uredinium of the disk of Gen I (cv. I-488) is replicated on that of cv. I-488 (Gen II) as a cluster of uredinia in a mirror image, however all these uredinia are not reproduced on disks of cv. T-173 (Gen II) suggesting that those which are misssing are avirulent on cv. T-173 (Uredinia 2 and 4 in Fig. A3.1). This avirulence was confirmed by producing mono-uredinial isolates from the

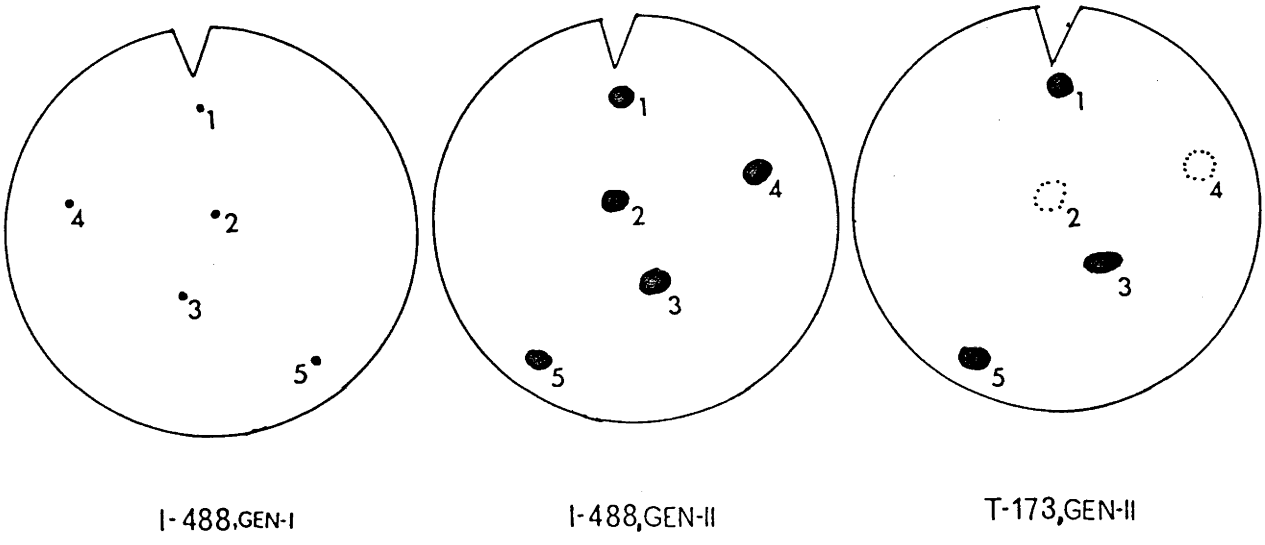


Figure A3.1 The leaf disk of susceptible cv. I-488 (Gen I) and the corresponding replica inoculated II Gen leaf disks of cvs I-488 and T-173 (resistant). All the uredinia of cv. I-488 (Gen I) are reproduced on cv. I-488 (Gen II) as a mirror image. On the disk of cv. T-173, missing uredinia nos. 2 and 4 would be avirulent on this cultivar and can be isolated from cv. I-488 (Gen II).

corresponding uredinia on cv. I-488 (Gen II) and re-inoculating disks of cv. T-173. The avirulent race isolated from this procedure is referred to as race 4B in the thesis. Similarly virulence of the isolates developing on disks of cv. T-173 (Gen II) was confirmed by re-inoculating disks of this cultivar (Isolates 4M1 and 4M2).

The technique was employed also to isolate temperature sensitive isolates of M. medusae from Race 4A which are virulent on P. deltoides cv. W-79/307 at 16° but avirulent at 26°. After several cycles of serial culturing at temperatures of 17, 20, 23 and 26°, a few uredinia developed at 26° (Chapter 10). Replica inoculations were made from the uredinia of this race on disks of this cultivar and one set was incubated at at 16° and another at 26°. Certain uredinial pustules which appeared at 16° did not appear at 26°, and thus could be recognised as characteristic of the original race 4A avirulent at the higher temperature. The isolate obtained from this procedure was referred to as race 4C.

SUMMARY

A technique to isolate avirulent isolates of leaf rusts from a mixture of virulent and avirulent isolates, employing a leaf replica method is described. The technique was also found useful in isolating temperature-sensitive isolates of the leaf rust.

APPENDIX 4

REVIEW OF LITERATURE ON GENETIC BASIS OF VARIABILITY FOR VIRULENCE SPECTRUM AND AGGRESSIVENESS IN FUNGI

Natural selection favours individuals and populations that acquire traits conducive to survival and reproduction. The generation of biological variation, which gives rise to new and potentially advantageous combinations of genetic traits, is therefore a central requirement for the successful evolution of all organisms in diverse and changing environments. Fungi are probably unique amongst eukaryotes in the variety of mechanisms by which they generate variability and maintain polymorphism. The basis of such variability has been reviewed by by many authors, including Day (1974), Webster (1974), Burnett (1975), Fincham et al (1979) and Caten (1982).

A4.1 SOURCE OF VARIATION IN FUNGI

A4.1.1 MUTATION : Mutation is the basic step in creation of genetic variability and is a sudden heritable change, which primarily involves changes in the nucleotide sequence. This assumption that mutation provides the fundamental basis underlying most of the observed variation in fungi is valid regardless of the rate with which mutations occur or the characters that are affected (Webster, 1974). Mutation affecting many traits have been demonstrated in rust fungi (Day, 1968 and 1974), while natural mutation from avirulence to virulence has been observed in many rusts (Flor, 1958; Watson, 1957; Samborski, 1963; Zimmer et al, 1963; Bartos et al, 1969; Pryor, 1977; Levitin and Mikhilova, 1981).

Such spontaneous mutants usually occur at low frequencies, and the natural mutation rate for virulence has been estimated to be around 1×10^{-8} to 1×10^{-3} per locus (Leyerstram, 1972; Day, 1978; Parlevliet and Zadoks, 1977). The size of fungal population ensures that mutant alleles arise continously and it is estimated that 10^4 to 10^{13} virulent

uredinia/conidia may appear every day per locus in a hectare of cereal crop infested with rust or powdery mildew (Day, 1978; Caten, 1982); this estimate has been suggested to be conservative (Person et al, 1976). Further, the observed mutation rates are affected by dominance or recessiveness of the virulence, homozygosity or heterygosity of the diakaryon when the virulence is recessive, and the penetrance and expressivity of the mutant allele.

The frequency of any given allele for virulence in a pathogen population is a function of the mutation rate that gives rise to the allele and the effect of the allele on the survival of the pathogen carrying it (Day, 1978). Mutation from avirulence to virulence can occur only on susceptible hosts, and the new virulence allele may apparently not be 'necessary' for the surivival of the pathogen on that host. However, if the new virulence allele confers fitness to the individual, because of the high asexual multiplication rates of many rust fungi, an initially rare allele can, under selection, increase in number and frequency very rapidly and may approach a frequency of 1.0. In contrast, if the allele reduces its fitness due to the possible genetic load associated with such mutation of the avirulence allele, its frequency will be close to the mutation rate (Day, 1978) and would usually go undetected. If the frequency of the resistant genotypes it can match is high and and if such genotypes exerts positive selection towards the new mutant, then its frequency would obviously increase.

Detailed annual virulence surveys of Puccinia graminis f. sp. tritici have shown many instances of single gene changes that were undoubtedly (McIntosh and Watson, 1982) the result of mutation. Similar observations were made by Green (1975) on the same pathogen in Candada for race 15B-1L group. While in some pathogens, some avirulence loci are known to be relativley unstable (Flor,1958, 1960; Watson, 1958; Day, 1974; Luig, 1978a), certain avirulence loci against some host genes have been postulated as relatively stable, since no natural mutants for virulence were observed nor could they be induced by chemical mutagen treatment of avirulent populations (e.g. Luig, 1978a).

Although mutations for a qualitative trait like virulence have been frequently recorded, precise studies on possible mutations affecting minor genes (genes with smaller effects) determining quantitative traits like aggressiveness are lacking. Variation for

quantitative traits is fairly well understood in certain fungi e.g. Pencillium, Saccharomyces etc., and has been exploited to increase productivity in industrial processes (Burnett, 1975). Differences in the aggressiveness traits, including latent period, sporulation and infection efficiency have been observed also among field isolates of plant pathogens (Clifford and Clothier, 1974; Johnson and Taylor, 1976; Parlevliet, 1977, 1983a; Hill and Nelson, 1982; Heather and Chandrashekar, 1982), and are considered to have high heritability (Hill and Nelson, 1981, Oard and Simons, 1983). It would be reasonable to assume that such differences would have a large genetic basis (Emara and Sidhu, 1974; Leonard, 1975). Although James and Fry (1983), detected changes in the aggressiveness of Phytopthora infestans after treatment with a chemical mutagen (NTG), directional selection for aggressiveness in strains by potato cultivar was not observed. Watson (1970) found that aggressiveness can be changed in some races of Puccinia graminis tritici by treatment with Ethyl Methane Sulphonate (EMS).

Although a few other (saprophytic) fungi have been observed to lose their virulence during artificial culturing (Day, 1974), studies on mutation to avirulence from a virulence allele, natural or induced, in rust fungi are inconclusive. Avirulent genotypes would be important for genetic and epidemiological studies (Day, 1974), and identification of such avirulent biotypes in an virulent population can be made employing suitable techniques (Appendix 3).

Griffiths and Carr (1961) reported that some virulent mutants of Puccinia coronata f. sp. avenae induced by UV rays, had lost their virulence on another other cultivar while Luig (1978a) observed that certain virulent mutants of P. graminis f. sp. tritici, upon subsequent treatment with a chemical mutagen (MNN), reverted back to avirulence. Green (1975) interpreted some changes in the race 15B-1L group of P. graminis f.sp. tritici in Canada, as mutation from virulence to avirulence.

A4.1.2 HETEROKARYOSIS : Heterokaryosis is the coexistence of genetically different nuclei in cytoplasmic continuity (Webster, 1974) and may arise by a number of methods including mutation in a multinuclear thallus, anastomoses, nuclear exchange between strains of closely related fungi or formation of multinuclear spores (Webster, 1974). Heterokaryosis may

be important in rusts due to the dikaryotic nature of the urediniospores. However certain authors feel that, as knowledge of heterokaryons is derived largely from laboratory studies, and because of the paucity of the confirmed examples of heterokaryosis in nature, its natural significance may be less than frequently assumed in the past (Caten, 1982).

A4.1.3 SEXUAL HYBRIDIZATION : Sexual hybridization is the major source of recombination in many rusts and thus the removal of alternate host populations contributes to reduced variability and often to reduced disease (Browning, 1973) e.g. the removal of Berberis vulgaris L. in the cereal growing regions of USA. Isolates or populations of Puccinia graminis f.sp. tritici from geographic regions where the sexual stage occurs have been observed to be more diverse than those collected from places where there is no sexual stage (Roelfs and Groth, 1980; Groth and Roelfs, 1982). However, Oard and Simons (1982), from a comparative study in laboratory of P. coronata populations, concluded that sexual mechanism may not confer significant advantages in generating quantitative variability. Lodge and Leonard (1984) suggested that asexuality may be advantageous in fungi because favourable gene complexes are not constantly broken by major recombinations, however, such a population would be at disadvantage when linkage disequilibria delay adaptation to complex new environments.

Although typically a heteroecious rust, the apparent absence in Australia of the aecial stage for Melampsora spp. suggests that observed variation can be attributed almost entirely to asexual processes. However, the wide spread occurrence of Pinus radiata D. Don. (recorded as an alternate host in the field for M. medusae in New Zealand; Van Kraayenrood, 1974) does not exclude the possibility of potential contribution of sexual hybridization to the variability in the gene pool in the field.

A4.1.4 SOMATIC HYBRIDIZATION : Rust fungi are known to undergo recombination resulting from hyphal fusions and nuclear exchange (Nelson, 1956) or from parasexual (mitotic) processes, despite the absence of sexual recombination. In Puccinia f.sp. graminis tritici,

Watson (1957) and Watson and Luig (1958) concluded that the multiplicity of variants arising from mixtures of urediniospores could result only from somatic hybridization. Other rust pathogens where asexual variation has been established include Melampsora lini (Flor, 1964), Puccinia striiformis (Little and Manners, 1969) and P. coronata (Bartos et al, 1969). It has also been shown that parasexual recombination in dikaryons of heterothallic basidiomycetes can occur through processes other than the parasexual cycle (Day, 1978, Frankel, 1979). At least one of these processess, meiotic like recombination, produces inter- and inta- chromosomal recombination at a frequency much higher than expected from the parasexual cycle (Frankel, 1979).

A4.1.5 CYTOPLASMIC FACTORS : A wide spectrum of morphological, physiological and biochemical variants have been identified in fungi in which their genetic control can be attributed to extrachromosomal mutation or activity of plasmagenes (Webster, 1974; Fincham et al, 1979). Such variation would be due to the presence of genetic systems in mitochondria and the occurence of stable, double-stranded RNA (ds RNA) molecules with independent genetic activity (Caten, 1982). In an analysis of many rust species, G. J. Lawrence and A. J. Pryor (Pers. commun.) detected the presence of ds RNA in eighteen of the twenty species tested. The ds RNA has been found to be associated with virulence in Endothia parasitica Fr. (Murr.) (Fullbright, 1984) and Rhizoctonia solani D.C. (Tavantzis and Bandy, 1984). Reports of extranuclear inheritance of factors conditioning aggressiveness or fittness in strains of P. infestans (Denward, 1970), Puccinia anomala Pers. (d'Olivera, 1940) and P. graminis f. sp. avenae (Green and McKenzie, 1967) suggest the importance of extrachromosomal variants in adaptive changes of the pathogen.

A4.1.6 TRANSPOSABLE ELEMENTS : Conventionally, the genome of an organism has been considered a stable entity but this belief is questioned by the discovery in Maize (McClincktock, 1956) and subsequently, in other organisms (Cohen and Shapiro, 1980), of mobile genetic elements which are bits of DNA that can move from place to place in an organsim genome turning other genes on and off (Federoff, 1984).

Although the activity of such transposable genetic elements (variously called jumping genes, control elements, insertion sequences, Ac - Ds system etc.) has not been conclusively demonstrated in plant pathogenic fungi, it's role has been implicated in a few systems. Luig (1978a) observed a high reversion rate from virulence to avirulence in a strain of P. graminis f.sp. tritici after treatment of virulent mutants types corresponding to the Sr 12 gene in wheat, with N-methyl-N-nitroso-N'-nitroguanidine (MNN). From this and other observations like reversiblity of colour mutants, (Luig, 1967), he concluded that such changes can be explained by assuming a position effect brought about by a control element. Transposable elements are now being increasingly used to probe certain bacterial pathogens, particularly in Pseudomonas syringae pv phaseolica through suicide plasmid vectors employed to carry Tn5 elements (e.g. Willis and Panopulos, 1984).

A4.1.7 UREDINIOSPORE MIGRATION : Although not a genetic factor per se, the migration of inoculum from areas where the uredinial stage can survive (overwinter) to other geographic areas, is a further potential source of variation in the racial composition of plant pathogens. Movement of cereal rust pathogens from southern USA, and probably less frequently Mexico, to northeren USA and Canada has been well discussed (Vanderplank, 1968). The introduction of Melampsora spp. to the Australian continent (Walker e al, 1974) and subsequent spread from the Colo region suggests the importance of urediniospore migration in the Populus - Melampsora system.

The mechanisms available to generate variation within the urediniospore population represent extreme flexibility for reassociation or recombination of nuclei, chromosomes, genes or cytoplasmic elements accompanying vegetative growth.

A race or biotype that increases in distribution and frequency among population of plant pathogens should possess, according to Nelson (1971),

(1) ability to incite disease (virulence)

(2) relative ability to cause greater amount of disease (agggressiveness)

(3) greater efficiency in causing disease

(4) ability to incite disease over a broader environment range

(5) increased ability to persist.

Hence, whilst variability is released as a consequence of predominantly asexual processes for Melampsora leaf rust in Australia, the subsequent expression of new effective traits in the pathogen population is dependent upon the reinforcement of these enhanced traits by positive selection by the host and the environment.

A4.2 GENETIC CONTROL OF VIRULENCE

Since the Goldschimdt (1928) demonstrated the genetic basis of virulence in the anther smut fungus (Ustilago violacae), several studies have been conducted to determine the inheritance of virulence or avirulence; these have been reviewed (Flor, 1971; Day, 1974; Burnett, 1975; McIntosh and Watson, 1982).

The pioneering work of Flor (1956, 1971) in the flax rust system revealed that inheritance of virulence was usually recessive to avirulence and there was no indication of multiple allelism in the pathogen, in contrast to the host where resistance was usually dominant and many multiple allele groups could be recognised. In a subsequent critical study of the same system, Lawrence et al (1981a) obtained evidence for a dominant inhibitor of avirulence for the M1 locus, and confirmed Flor's studies indicating that genes AP1, AP2 and AP3 in M. lini are sufficiently closely linked to suggest that they may be allelic (Lawrence et al, 1981b). Similar linkage of virulence loci has been observed or suggested in other fungi (Luig, 1968b; Sambroski and Dyck, 1968; Statler, 1979; Gustaffson, 1984). Dominance of virulence alleles has also been noticed in few instances (Luig and Watson, 1961; Green, 1966; Sambroski and Dyck, 1968).

Generally, studies have revealed a simple inheritance of virulence genes in plant pathogens

A4.3 GENE-FOR-GENE RELATIONSHIP

The gene-for-gene hypothesis has been extensively employed to explain the relationship between host and pathogen, both from the practical and evolutionary points of view (Flor, 1971; Mode, 1958; Person, 1959; Person and Sidhu, 1971; Day, 1974; Parlevliet and Zadoks, 1977; Ellingboe, 1978).

Flor's inheritance studies (1956, 1971) showed that in order to overcome each unique resistance gene in the host, the pathogen must carry a matching virulence gene, and Flor (1956) concluded that 'for every gene controlling resistance in the host, there is a corresponding gene in the parasite controlling pathogenicity'. Two virulence genes are needed in the pathogen to counter two resistance genes in the host (Person, 1959) and so on; thus a numerical equivalence in genes for a/virulence in the pathogen and of resistance in the host were observed and such genes were called corresponding or complementary genes. The relationship usually observed for biotrophic pathogens, when presented on a quadratic check (Rowell et al, 1963) (e.g. Table 6.1) indicates that incompatibility occurs when a host resistance gene and a corresponding avirulence genes interact. Ellingboe (1978) and Parlevliet (1983a) have suggested that such a relationship indicates that recognition in the system is for incompatibility, and resistance - avirulence are the active alleles, with such a system superimposed on basic compatability system. The gene-for-gene relationship has been demonstrated or suggested to operate in many pathosystems (Person and Sidhu, 1971; Day, 1974; Sidhu, 1975).

The relevance of the gene-for-gene relationship in explaining the qualitative specificity between hosts and pathogens is obvious. However there are conflicting opinions of the possible operation of such a relationship in a polygenic system i.e., between aggressiveness genes in the pathogen and for quantitative resistance/susceptibility genes in the host. While some workers suggest that gene-for-gene relationship may not be operative in a polygenic system and thus such a system would be predominantly non-specific (Vanderplank, 1968; Person et al, 1983), others suggest that gene-for-gene relationship may be operative for polygenic traits (Parlevliet and Zadoks, 1977; Ellingboe, 1978). The latter view is supported by the differential interaction of host-

pathogen lines for quantitaitve traits like latent period and sporulation, sometimes accompanied by a reversal in ranking (Johnson and Taylor, 1976; Scott et al, 1980; Heather and Chandrashekar, 1982). Although these reports are not direct evidences for gene-for-gene relationship, they are compatible with such a hypothesis. Parlevliet (1983a) suggests such quantitative specificity is the expression of basic compatibility system between polygenes for aggressiveness in the pathogen and for partial resistance in the host. However, existence of the gene-for-gene relationship for polygenes may never be conclusively demonstrated (Parlevliet and Zadoks, 1977) mainly due to the inherent complexities involved in the quantitative system, and the occurrence of nonheritable variation.

While gene-for-gene relationship has been suggested (Person, 1959) or implied (Ellingboe, 1978) to be of general occurrence rather than exception, certain workers, notably Day (1968, 1974), Day et al (1983), Barrett (1983), while accepting the importance of the theory in explaining the relationship between qualitative virulence and resistance, contend that the gene-for-gene hypothesis may be an oversimplification of complex events occurring in the pathosystem. They feel that such a single theory, 'which may be the tip of the iceberg', and may be an artefact of breeding for resistance, would not explain all forms of resistance including host variations affecting partial or incomplete resistance, slow rusting, genotype-environment interaction and the possible effects of virulence allele on aggressiveness, the role of modifiers and background effects of both host and pathogen genotypes. Similar views have been expressed by Watson and Luig (1968a), Wolfe and Schwarzbach (1978) and Johnson (1983).

A4.4 GENETIC CONTROL OF AGGRESSIVENESS

In contrast to virulence, literature on the genetic control of aggressiveness is sparse, although this component may determine the severity of a disease epidemic or the potential spread of a newly arisen race (Caten, 1982). Possibly, this results from lack of appreciation of the importance of this component (Nelson, 1979) or because of the complexities involved in dealing with a quantitative trait. Nelson

(1979) remarked 'failure to monitor changes in parasitic fitness is one of the major reasons why the plant breeder never released a resistant variety **before** the damage was done' (emphasis by him). Similarly Pryor (1977) and Wolfe and Schwarzbach (1978) have outlined the need for greater emphasis on aggressiveness in the pathosystem studies. Studies on aggressiveness need be focussed on differences in aggressiveness, both between the races and between the biotypes within a race, because as Day (1978) commented, it would be a fallacy to think of physiologic races in nature as fixed clonal entities.

As a quantitative character, aggressiveness may show continuous distribution and thus, would expected to be polygenically inherited; this expectation is supported by few studies. For example, the higher aggressiveness in Ceratocystis ulmi (Buismi) C. Moreau is determined by a complex polygenic system (Brasier, 1977). Aggressiveness in strains of Ustilago hordei (Pers.) Lagerh. differ in their infective ability on compatible barley cultivars and the variation in this trait was determined by a polygenic system, with possible dominance of higher aggressiveness (Emara and Sidhu, 1974). Leonard (1975) also observed polygenic inheritance of lesion size trait in Cochliobolus carbonum Drechs.

Differences in aggressiveness between strains have been recorded in many plant pathogens (Katsuya and Green, 1967; Clifford and Clothier, 1974; Leonard, 1969, 1977; Martin and Ellingboe, 1976; Hill and Nelson, 1981; Heather and Chandrashekar, 1982), and some studies have shown that aggressiveness, like virulence, can exhibit host-specificity (Clifford and Clothier, 1974; Caten, 1974; Johnson and Taylor, 1976; Triantophyllou, 1975; Martin and Ellingboe, 1976; Hadley et al, 1979 ; Wolfe and Knott, 1982; Chandrashekar and Heather, 1983; Singh and Heather, 1983b; Wolfe et al, 1983; Leonard, 1984), and some of these studies demonstrated that isolates originating from a particular host were more aggressive on those hosts compared to others. However, Vanderplank (1978, 1982) maintains that aggressiveness is basically a non-specific character, and suggests that these minor variations observed may be artefacts having very little epidemiological consequence.

A4.5 RELATIONSHIP BETWEEN VIRULENCE AND AGGRESSIVENESS

Virulence is considered here as the ability of an isolate of the pathogen to attack and successfully reproduce on a given host (IT 2-4), while the virulence spectrum is the number of cultivars, within a set of host cultivars, on which the isolate is virulent. Aggressiveness is the relative amount of disease caused by the isolate on a particular host cultivar or cultivars on which it is virulent, and is a quantitative expression of the disease; thus it reflects the fitness of the isolate. Hence, an isolate can only be aggressive on a cultivar on which it is virulent although differences in aggressiveness can be expected amongst the virulent isolates on the same and between different compatible cultivars. The relationship between the virulence spectra and the aggressiveness of isolates, has been widely discussed (Vanderplank, 1968; Watson, 1970; Brown, 1975; Nelson, 1979; Crill, 1977, Parlevliet, 1981a). Virulent mutants, that are a threat to newly introduced, resistant cultivars will arise in a pathogen population maintaining itself on a susceptible host (Person *et al*, 1976). Their fitness on the susceptible hosts is important in determining the frequency of such mutants; this accounts for the interest in the effect of unnecessary genes for virulence in a pathogen population. Person *et al* (1976) have discussed the possible consequences of newly arising virulent mutants on susceptible hosts, in the presence or absence of the corresponding resistance genes in the population.

Black (1952) and Thurston (1961) found that strains of *P. infestans* with wider virulence spectra were less aggressive on simple cultivars (*sensu* possessing few genes for resistance) compared to those with narrow virulence spectra. A similar relationship was observed for flax rust by Flor (1971) and for wheat stem rust by Watson (1958) under field conditions. On the basis of the data available at the time, Vanderplank (1963, 1968) introduced the concept of 'stabilising selection' according to which there was an inverse relationship between certain alleles for virulence and the relative fitness of pathogenic isolates to survive, when such alleles were unnecessary for survival in

host populations. He further suggested that an increased virulence (sensu virulence spectrum) is associated with decreased fitness and proposed that in certain situations such 'unnecessary' virulence still persisted in populations because of the 'weak' host resistance genes, to which the corresponding virulence loci in pathogen were not fully unfit to survive even though unnecessary (Vanderplank, 1968, 1975).

Due to the implications of the 'stabilising selection' theory in the evolution of the host-pathogen systems and in disease management strategies (Van der Plank, 1968, 1978; Leonard, 1977; Leonard and Czochor, 1980), several workers attempted to test the validity of this theory by, i) determining the frequency of 'unnecessary' virulence genes in field populations of the pathogen, ii) inoculating the compatible cultivars with a mixture of races differing in virulence spectra and evaluating the dominance of strains in such a competitive situation over few generations, or iii) independently evaluating aggressiveness of such races. While some studies revealed that races with some 'unnecessary' virulence were less competitive or less frequent (Leonard, 1969 and 1977; Scheifele et al, 1968, Grant and Archer, 1983), other studies showed exceptions to this (Watson, 1970; Ogle and Brown, 1971; Flor, 1971, Osoro and Green, 1976; Gustaffson, 1983). However, as pointed out by Leonard and Czochor (1980), many studies conducted to test the selection pressure against 'unnecessary' virulence allele do not meet certain requirements viz. testing the fitness of unnecessary virulence alleles without the confounding effects of differences in background genotypes of the pathogen, and they suggested the use of isolines of the pathogen for such precise studies. In a few studies where these criteria were generally met, then the 'cost of unnecessary virulence' was found to be 0.12 to 0.40 (Leonard, 1977; cf. Watson and Singh, 1952) although Osro and Green (1976), Bronson and Ellingboe (1981) could not observe such 'cost of virulence'. Similarly many workers have stressed the need for using isolines in the pathogen to evaluate the effect of differences in virulence, on the aggressiveness traits (Rowell et al, 1963; Day, 1966; Leonard, 1969; Osoro and Green, 1976).

Leonard and Czochor (1980) have pointed out that the term 'stabilising selection' is misleading in the present context, as such a selection operates against extremes at the either end of the scale for a

trait and tends to normalise the population (in population genetic sense) and thus the term 'selection against unnecessary virulence' would be better.

Thus, although the finding of Nelson et al (1970) and Singh and Heather (1983), that some isolates with wide virulence spectra were generally more aggressive on the cultivars tested, is at variance with Vanderplank's (1968, 1975) suggestion that virulence (spectra) would be negatively associated with aggressiveness, they are not in conflict with the theory of Leonard (1977) and Leonard and Czochor (1980), because the virulence spectra tested in the above two studies were 'necessary' for the infection on those cultivars tested. In their models, Leonard (1977) and Leonard and Czochor (1980) showed the importance of the deleterious effects on fitness of such increased 'unnecessary' virulence (cost of virulence) in the co-evolution of host-pathogens, and for maintaining stability in host-pathogen systems through balanced polymorphism. Similar findings were made by Marshall and Pryor (1978) on the importance of 'cost of virulence' in disease control by multiline strategy. Parlevliet (1981a), while accepting the possible role of such a selection in natural pathosystems, suggested that 'stabilising selection' (sensu Vanderplank, 1968) may not be an important factor in agricultural pathosystems where man controls host genotype patterns over time and space. However, he (Parlevliet, 1983a) has suggested that in host-pathogen coevolution, the selection is towards moderate virulence, which may be attained by the compromising forces of Darwinian selection for increased aggressiveness, and the group selection for avirulence, as both extremes are detrimental to the host-pathogen systems. He implies that, in an biotroph, the avirulence allele, despite being an altruistic trait to the individual, is selected, not because of the fitness associated with it, but because of the feedback system in the population where high virulence, can endanger the survival of the host, thus endangering survival of the pathogen. Wolfe et al (1983) have detailed the relevance of interaction of adapatation (increased aggressiveness on specific hosts by specific isolates) and adaptability (increased virulence spectrum) in crop pathosystems, and provides experimental evidence that pathogens make a compromise between these two components which are strongly determined by the extent of the host genotype. Thus, in the beginning of the infectious season, the selection is towards

adaptability (increased virulence spectrum), which ensures maximal infection efficiency for the population by being flexible in its choice of host genotypes, followed by a phase of increased adaptation by the isolates on cultivars on which they harbour. He advocates employing cultivar mixtures as an effective disease control strategy which would keep both adaptation and adaptability in check.

Many workers have observed that current theories tend to simplify the relationship in theoretical studies, and further, studying the independent effect of individual factors, they tend to ignore the interactive features involved (MacKey, 1981; Heather and Chandrashekar, 1982). The simplicity of the gene-for-gene hypothesis has facilitated the development of explicitly genetic models of host-parasite coevolution. However Barrett (1984) observed 'indeed, it is probable that even had the gene-for-gene hypothesis not existed, it would have been necessary for population geneticists to invent it in order to model host-parasite systems', and remarked that models based on gene-for-gene relationships have considered only genetic changes and neglected changes in population size and other ecological factors. For example, Brown (1975) identified following factors, in addition to host factors and selection against avirulence alleles, as likely to affect the relative ability of strains of fungal pathogens to survive in populations,

(1) Variation in aggressiveness traits.
(2) Existence of environmental ecotypes.
(3) Indirect effect of environment owing to its effect on the host resistance.
(4) Interaction between strains within a population.

A4.7 COEVOLUTION OF THE HOST-PATHOGN SYSTEM

The host - pathogen pair has been called the 'odd couple' of the biological world, and their coevolution a spectacular biological saga by Nelson (1979). Biologists have studied intensively and speculated on the coevolution of the host-pathogen system, although it is limited to annual hosts.

The hosts tend to evolve defence mechanisms against the pathogen and in turn, pathogens acquire virulence to overcome the defence, and this reciprocal behaviour leads to a spiral of an evolutionary arms race. The evolution of the host-pathogen system is influenced also by the physical environment, and in agro-ecosystems, by man. Selection results from differences in fitness among individiuals of a population (Mettler and Greg, 1969) and fitness is mainly a measure of the reproductive success of individuals,i.e. those biotypes which survive best and pass on their genes to the greatest nunmber of viable progeny are the most fit (Leonard, 1977). Those genes that enhance the fitness of individuals of particular population will tend to increase in frequency in that population, the process referred to as selection. The selection can be for traits at one end of the scale (directional), can be at many ends of the scale (disruptive) or around the mean of the scale (stabilising)] (Mather, 1955; Thoday, 1972). Also, other forms of selection include those due to heterosis, and those that are frequency-dependent e.g. the organism is most fit when it is least frequent (Ayala and Valentine, 1979; Futuyma, 1979).

The central theme in host-pathogen coevolution is that, although the fitness of host lies in successfully warding off the pathogen or minimising the pathogen's effect on its fecundity, fitness of an obligate parasite depends not only on its ability to survive and reproduce, but also to ensure survival of the host, or at least not to endanger its survival. This association would be even more critical in a perennial host-biotroph pathogen situation such as the poplar leaf rust system.

While the rate at whcih coevolution takes place varies, there are several models which explain how the stability may be achieved in the pathosystem. Mode (1958) made the first theoretical analysis of genetic interactions between populations of plants and their pathogens in a gene-for-gene relationship and described a set of mathematical conditions under which a stable equilibrium can be reached in the system. Leonard (1977), in a subsequent critical analysis, showed the importance of variables viz. cost of virulence, effectiveness of resistance, and the advantage of virulent race on a host with the corresponding genes for resistance, in attaining a stable equilibrium by balanced polymorphism of the hosts and pathogens. Leonard and Czochor

(1980) suggested that balanced polymorphism, as opposed to transient polymorphisms, constitutes the majority of observed examples of polymorphism in the gene-for-gene systems, because they persist and can accumulate, in contrast to transient polymorphism which by definition is temporary.

Parlevliet (1983a), in explaining the host-pathogen coevolution, suggested that recognition systems for incompatibility and compatibility phemomena are important in a pathosystem, and in the barely rust system, the former was characterised by hypersensitive type resistance and the latter by partial resistance. Assuming both systems operate on a gene-for-gene basis (Parlevliet and Zadoks, 1977), he suggested that virulence is regulated to intermediate levels by the opposing forces of Darwinian (favouring high aggressiveness) and group selections (favouring high avirulence) in the incompatible relationship. The compatible relationship tends to be relatively more stable, because the recessivenes of the low aggressiveness necessitates a gain mutation, which is not easy to produce, to neutralise the effect of the resistance gene. The incompatibility system is superimposed on the compatibility system (Ellingboe, 1978) and thus regulates the coexistence of the pathogen with the host at intermediate levels. Heath (1981) has discussed the physiological and biochemical implications of these two systems.

Many other models have been proposed, some with direct relevance to disease control strategies (Mode, 1961; Groth, 1976; Marshall and Pryor, 1978, 1979; Barrett, 1980; Wolfe et al, 1983) and a critical review of some of these models have been made (Leonard and Czochor, 1980). Similar works on general host-predator relationships have been attempted (e.g. Futuyama, 1979). The results of most models indicate that stable or dynamically stable polymorphism can be maintained by reciprocal frequency-dependent selection as predicted by Haldane (1954) (Barrett, 1983).

Most of the above studies assume a specific interaction based on the gene-for-gene relationship in exlaining the plant-pathogen coevolution and modes of achieving stability. In contrast, some authors have suggested non-specific interaction (sensu absence of differential

host-pathogen interaction) based primarily on polygenes (Person et al 1983) would contribute to the stability of the pathosystem, and this would be achieved in an additive model which would predict constant ranking, coupled with 'phenotypic damping' and opposition of selective forces operating in the two interacting populations.

It is becoming clear that man's role as a component of the disease square is increasing and future orientation of the sytem now largely rests with him. Nelson (1979) summed the future aptly 'If man is to be the director of their coevolution let him see to it that they play in harmony'.

APPENDIX 5

EFFECT OF GAMMA IRRADIATION ON GERMINATION OF UREDINIOSPORES OF *MELAMPSORA MEDUSAE*

Estimation of the radiosensitivity of organisms is a prerequisite for establishing dosage limits for programmes on induced mutation. In certain fungi, spore germination has been employed successfully as a criterion to assess infection potential after irradiation (Kwon and Oh, 1977). This preliminary study compared the capacity of urediniospores of *M. medusae*, which have been exposed to increasing levels of gamma irradiation, to germinate and to infect leaf disks of *P.* x *euramericana* cv. I-488.

Two races 1A and 5A of *M. medusae* were employed in the study. Individual samples of fresh spores (*c.* 80% spore water content) were exposed to a ^{60}Co gamma source to obtain 0, 200, 400, 1000, 1500 and 2000 Gray (Gy) (1 Gy=10 rads) dosages of irradiation and subsequently dried over silica gel (12 h) and P_2O_5 (12 h) to reduce the spore water content to *c.* 20 %. Urediniospores (5 mg per dose-treatment) were deposited on five replicate cover glasses (1.32 cm^2) and 20 leaf disks (1.70 cm^2) of *P.* x *euramericana* cv.I-488, a universal suscept, in a spore settling tower (section 2.4.2). Spore germination was assessed on the coverglasses which were placed on plastic foam soaked in sterile water, sealed in sterile Petri dishes to ensure 100% relative humidity, and incubated under diffused light (50 uEm-2s-1) at 16 ± 1 °C for 24 h (Singh and Heather, 1982c). Spores were considered to have germinated if the germ tube was at least as long as it was wide.

The inoculated leaf disks of cv. I-488, placed on plastic foam soaked with GA located in Petri dishes, were incubated in controlled growth cabinets at 'standard environment' (section 2.5.1). The number of uredinia produced per leaf disk (**ULD**) was counted after 18 days and the infection potential of the irradiated spores was expressed as a percentage of the number of uredinia induced by the control (0 Gy)

urediniospores. The analyses of variance (**ANOVA**) for germination and uredinial number (ULD) used angular and square root transformations, respectively. For the urediniospores of the two races, germination percentage (untransformed values) on the coverglasses and infection potential (percentage) on leaf disks, at levels of irradiation from 0 - 2 X 10^3 Gy, are presented in Fig. A5.1.

In the two way ANOVA for germination, the major factors (dosage of irradiation and races of the pathogen) but not their interaction component were significant ($P < 0.01$) determinants of variation (ANOVA not presented). At all dosage levels, race 5A was relatively more radioresistant than race 1A (Fig. A5.1). Although in both races, germination decreased moderately at levels of irradiation below 400 Gy, it increased in the range 400 to 1000 Gy (significant in race 1A, $P < 0.01$) and even at 2000 Gy it exceeded 65% (Fig. A5. 1).

In the ANOVA for ULD, the dosage of irradiation, but not the race of the pathogen, was a significant ($P < 0.01$) determinant of variation while the interaction component, dose X race, was also a significant ($P < 0.01$) contributor to such variation. The infection potential of both races decreased almost linearly with increasing radiation levels over the range 0-1000 Gy and approached zero at the latter level. At all dosages of irradiation, the infection potential of treated urediniospores of race 5A was higher than that of race 1A.

Germination of the urediniospores of both races of M. medusae exceeded 65% after exposure to irradiation levels greater than 1200 Gy; however spores exposed to such levels did not successfully infect leaf disks. Hence the capacity of these spores to germinate following irradiation was not a suitable criterion of infection potential. When examined with a scanning electron microscope, spores treated at 2000 Gy had normal germtubes. These observations agree with Schwinghamer (1958) who found that only doses of 10000 Gy of X or Gamma rays completely inhibited germination of urediniospores of M. lini. He concluded that such high dosages might inactivate cytoplasmic components.

The nucleus is the prime target of gamma irradiation and loss of infection potential in exposed cells is primarily a consequence of

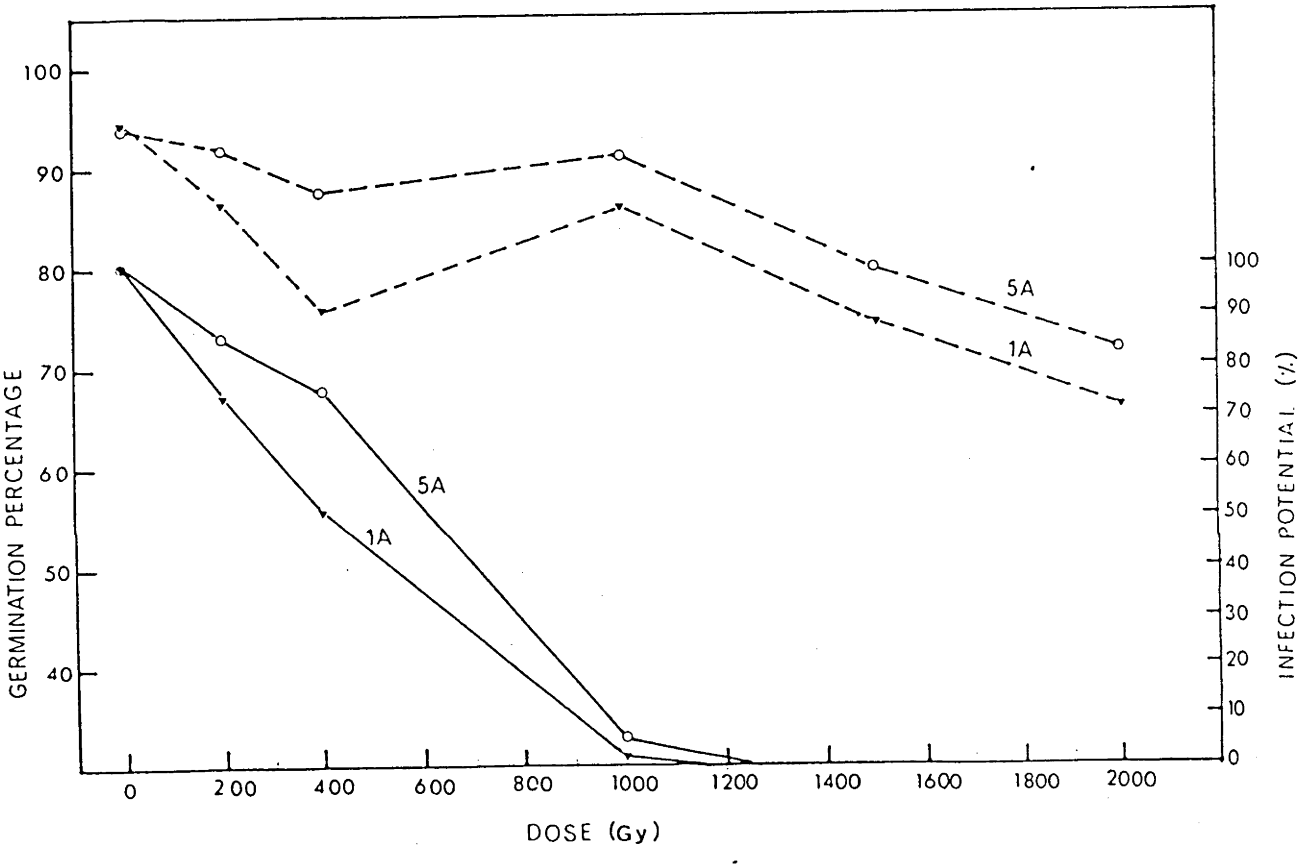


Figure A5.1 Mean germination percentage (- - - -) on coverglasses and mean infection potential (---------) on leaf disks of P.x euramericana cv. I-488 of the urediniospores of two races of M. medusae. Samples of these spores had been exposed previously to various dosages of gamma irradiation.

damage to nuclear DNA (Davies and Evans, 1966). The DNA and enzyme complement involved in the germination of urediniospores of M. medusae are possibly synthesised during the spore maturation cycle, hence inactivation of nuclear DNA by irradiation of mature spores does not affect their germination. Such an observation would agree with Brody (1982), who concluded that early events in the germination of Neurospora were 'preprogrammed' into the conidium during its formation and with Staples (1974), who showed that DNA synthesis and functional nuclei were not needed during the germination process in Uromyces phaseoli Pers.

SUMMARY

Urediniospores of two races of M. medusae were exposed to increasing levels of gamma irradiation, and their germination on coverglasses and infection potential (uredinial survival) on leaf disks of P.x euramericana cv. I-488, were assessed. The germination was not a suitable criterion to assess radiosensitivity as there was good germination at higher levels of irradiation while the infection potential at these levels was very low.

APPENDIX 6

INDUCTION OF RAPID AND SYNCHRONOUS GERMINATION OF UREDINIOSPORES OF *MELAMPSORA MEDUSAE* FOR ELECTROPHORETIC STUDIES

Electrophoretic techniques are being increasingly used in the identification of the fungal species, to investigate the intra-specific variation in the fungi, and have potential in the population genetic studies (Burdon *et al*, 1982). Synchronous germination of large masses of urediniospores is a pre- requisite for the application of the gel-electrophoresis technique in the study of variation in the rust fungi (Burdon *et al*, 1982). A surfactant, Tritron X-100, added to spore suspensions and shaken vigorously, removed inhibitors and resulted in germination of large masses of spores in many rusts and smuts (Schipper *et al*, 1969). Maheshwari and Sussman (1970) obtained almost complete germination of *P. graminis tritici* by suspending the urediniospores in a calcium phosphate-potassium phosphate buffer (pH 7.0) containing 1x10-4M nonyl alchohol and 0.01% Tween 20.

In both species of poplar leaf rusts, *M. medusae* and *M.larici-populina*, trials with the methods cited did not result in satisfactory levels of germination (Prakash, unpublished; Rayner, 1983). Germination in suspensions of low density rarely exceeded 5%; when placed on membrane filters, only individual spores germinated with no germination among clumped spores. Spores in these rusts are formed separately and clumping is primarily due to the echinules on the spore walls. Although germination of more than 80% has been reported earlier for spores of *M. medusae* when exposed on coverglasses in 100% R.H. (Singh and Heather, 1982; section 2.4.2; Chapter 2), these figures were derived from counts of individual spores and ignored the occassional clumps. Such observations, which describe the 'germinative potential' of the spores, are appropriate to epidemiological studies but are unsuitable for studies on physiology of fungal spores. Nicotine (100 ul l-1) which enhanced the germination (>70%) of spores of *M. larici-populina* in

suspension (Rayner, 1983), increased vacuolation in some fungal spores (French and Wilson, 1981) and may affect the enzyme systems. Thus, a technique was developed to obtain synchronous and en masse germination of the urediniopsores of M. medusae, for possible subsequent electrophoretic application.

The inoculum of races 1A and 4A of M. medusae were used in this study. The procedures on multiplication of spores on detached leaves of P. x euramericana cv. I-488 under controlled conditions of light, temperature and photoperiod followed as detailed in Chapter 2.

Dry spores were placed in sterile McCartney bottles (1 mg /bottle, ca. 6 X 10^4 spores) containing 10 ml of monobasic calcium phosphate (10^{-4}M)-mono and dibasic potassium phosphate (10^{-2}M) buffer,pH 7.0 (Maheshwari and Sussman, 1970). The suspension was homogenised for 30 sec using a high-frequency disperser (Ultra-turrax®, TP 18/10 shaft, ca.10^4 rpm, Janke and Kunkel KG, ika-werk, Staufen, West Germany) and subsequently poured into a vacuum filter assembly containing a membrane filter (Metricel® , diameter 47mm, pore size 5 um, Gelman Filter Co. U.S.A.). The suspension was swirled and a vacuum (-70 KPa) drawn for 2 min, allowing the urediniospores to deposit evenly on the filters (Schipper, et al, 1969) which were placed on Whatman No.1 filter paper (two layers), moistened with 2ml sterile water in 9 cm Petri dishes. The use of more than 2 ml of water per filter disturbed the uniform spread of spores and hence affected germination. The Petri dishes were given a cold-treatment of 4°C for 60 min and subsequently incubated at 16±1°C (Singh and Heather, 1982) under diffused light (50 $uEm^{-2}s^{-1}$). The cold treatment enhanced synchrony of germination.

In this method the spores were uniformly distributed over the filters without any clumping and germination of more than 95% was regularly obtained in successive tests. In most spores initial germtube protrusion could be observed after 30 min incubation and complete germination (germtube being as long as it was broad) was achieved in 4 hours.

When sterile water, buffer with $1x10^{-4}$ M nonyl alchohol and Tween 20, or nonyl alchohol alone was used, germination was always lower than with buffer solution alone. Incubating the spores in darkness did

not enhance germination signficantly when compared to diffused light. In several trials, incubating in alternate light and dark fluctuation cycles (5-5 min) increased germination significantly but these effects were not be consistently reproduced.

When many races of M. medusae were assessed for isoenzyme variation (with the assistance of Dr. J. J. Burdon, CSIRO Division of Plant Industry), those employing germinated urediniospores from the above technique had better electrophoretic activity compared to that with ungerminated spores (Prakash, unpublished). Also, pronounced differences in the isoenzymes were observed between races with narrow and broad virulence spectra (i.e. the wild types and the virulent mutants). The latter races had lost activity for some alleles in few isoenzymes, but had exhibited activity for some alleles with in certain isoenzymes, when compared to the avirulent wild types (Prakash, unpublished). The acquisition of new alleles by the mutants was surprising, but however support the regulatory gene theory proposed in Chapters 5 and 15. Such studies may have significant potential in identification of virulence or avirulence alleles in the pathogen, and for possible isolation of gene products involved in these function.

SUMMARY

A technique to induce rapid and synchronous germination of large masses of urediniospores of melampsora medusae is detailed. It involves homogenising the spore suspension in buffer using a high frequency desperser followed by uniform deposition of spores on a membrance filter through vacuum suction and subsequent incubation on a moist filter paper in a Petri dish. A uniform germination of more than 95% was obtained repeatedly by this method which is useful in electrophoretic and other physiological studies of the rusts.

APPENDIX 7

A7.1 THE DATA ON THE STUDY OF DISASSOCIATION OF AGGRESSIVENESS AND SURVIVAL IN MIXTURES OF RACE 4M OF MELAMPSORA MEDUSAE

Table A7.1 Summary of the variances[a] of the race 4B and 4M, and their mixture of M. medusae for three aggressiveness traits[b] representing three generations (1, 6 and 11) on cvs I-488 and T-173.

Source	D F	LP1	ULD[c]	USM[c]
Race	3	27.33	25.01	28.07
Generation	2	9.12	6.32	0.56ns
Cultivar	1	766.09	1017.75	366.41
Race X Generation	5	8.22	13.07	7.08
Race X Cultivar	3	25.01	9.02	33.25
Generation X Cultivar	2	7.81	6.72	0.17ns
Race X Genr X Cultivar	5	9.86	12.56	4.78
Residual	308	0.63	0.45	0.57d
Total	329	4.05	4.63	2.86[d]

a Mean sum of squares has been presented. All unmarked values are significant ($P < 0.001$); * denotes significant with $P < 0.025$; 'ns' denotes not significant.

b The three aggressiveness traits are latent period to production of first uredinium (LP1), uredinia produced per leaf disk (ULD; square root transformed) and urediniospores produced per square mm of leaf (USM; Log + 1 transformed).

c Using transformed values.

d The residual and total degrees of freedom for USM is 241 and 262 respectively.

Table A7.2 Meansa for three aggressiveness traits on cvs I-488 and T-173 by virulent race 4M (natural virulent mutant) of M. medusae cultured cv. I-488 (4M-I488) and on cv. T-173 (4M-T173), assessed after 1, 6 and 11 generations of serial culture. The 6 generation isolate of race 4M-T173 was not studied. The values sharing the same alphabet within a row do not differ significantly (P > 0.05). For comparisons of values across rows, use Least Significant Difference (LSD) values indicated under each trait.

a) **LATENT (DAYS) TO PRODUCTION OF FIRST UREDINIUM (LP1)**

	REACTION ON cv. I-488			REACTION ON cv. T-173		
GENERATIONS	1	6	11	1	6	11
RACES						
4M-I488	8.93b	8.40a	8.28a	10.73c	11.64d	11.33d
4M-T173	8.93a	-	9.53b	10.73c	-	10.20c

Least significant difference (LSD)0.05 = 0.570

b) **UREDINIA PRODUCED PER LEAF DISK (ULD)** (using square root transformed values)

	REACTION ON cv. I-488			REACTION ON cv. T-173		
GENERATIONS	1	6	11	1	6	11
RACES						
4M-I488	2.82c	3.53b	3.92a	2.77c	3.50b	4.15a
4M-T173	2.82b	-	2.83b	2.77b	-	3.55a

Least significant difference (LSD)0.05 = 0.568

c) **UREDINIOSPORES PRODUCED PER SQUARE MM OF LEAF (USM)** (using Log + 1 transformed values).

	REACTION ON cv. I-488			REACTION ON cv. T-173		
GENERATIONS	1	6	11	1	6	11
RACES						
4M-I488	5.69a	6.05a	6.10a	5.13b	3.80c	4.34c
4M-T173	5.69ab	-	5.95a	5.13b	-	5.03b

Least significant difference (LSD)0.05 = 0.607

[a] mean of fifteen replicates.

Table A7.3 Means for three aggressiveness traits by race 4B and race mix 4B + 4M of M. medusae on cvs I-488 and T-173, assessed after 1, 6 and 11 generations of culturing on cv. I-488. The values sharing the same alphabet within a row do not differ significantly ($P < 0.05$). Use appropriate LSD values from Table A.7.2 for comparisons across rows.

a) **LATENT PERIOD TO PRODUCTION OF FIRST UREDINIUM (LP1)**

	REACTION ON cv. I-488			REACTION ON cv. T-173		
GENERATIONS	1	6	11	1	6	11
RACES						
4B	9.27b	9.13b	8.40a	13.66e	12.82d	11.27c
4B + 4M	9.53b	9.00a	8.73a	11.50c	13.91e	13.26d

b) **UREDINIA PRODUCED PER LEAF DISK (ULD)** (using square root transformed values)

	REACTION ON cv. I-488			REACTION ON cv. T-173		
GENERATIONS	1	6	11	1	6	11
RACES						
4B	4.96b	5.12b	5.77a	0.84d	2.03c	2.46c
4B + 4M	3.28a	2.94a	2.29b	1.94c	1.49d	1.88cd

c) **UREDINIOSPORES PRODUCED PER SQ. MM OF LEAF (USM)** (using log + 1 transformed values)

	REACTION ON cv. I-488			REACTION ON cv. T-173		
GENERATIONS	1	6	11	1	6	11
RACES						
4B	6.70a	6.70a	7.02a	2.17c	3.79b	4.28b
4B + 4M	5.96a	5.91a	5.41a	2.67b	2.96b	1.43c

a mean of fifteen replicates

A7.2 REACTION OF RACES AND RACEMIXES ON TWELVE CULTIVARS OF POPLARS

Table A7.4 Reaction of twelve cultivars of poplars to serially cultured races and racemixes of *M. medusae* (from Chapter 8; involving radiation induced virulent mutant) as assessed by infection type[a] (0-4 scale of increasing disease severity).

			GENERATION	
	CULTIVAR	RACE	1	6	11
P. deltoides	cv. W-79/304				
		5A	1	1	1
		5M-I488	2	2	2
		5M-T173	2	2	1
		5A + 5M	1[a]	1[a]	1[a]
	cv. W-79/306				
		5A	2[b]	2	2
		5M-I488	3	3	3
		5M-T173	3	3	3-4
		5A + 5M	2	2	2
	cv. W-79/307				
		5A	2	2	2
		5M-I488	3	3	3
		5M-T173	3	3	4
		5A + 5M	2	3	3
	cv. 7-2	5A	3	2	2
		5M-I488	0	0	0
		5M-T173	0	0	0
		5A + 5M	2	1	1
	cv. 7-4				
		5A	2	2	2
		5M-I488	2-3	3	3
		5M-T173	2-3	3	3[b]
		5M-T173	2[b]	2	2
	cv. 10-3				
		5A	0	0	0
		5M-I488	0	0	0
		5M-T173	0	0	0
		5A + 5M	0	0	0
	cv. 60/164				
		5A	0	1-2	2
		5M-I488	4	3-4	4
		5M-T173	4	4	4
		5A + 5M	2	2-3	2

CULTIVAR	RACE	GENERATIONS 1	6	11
cv. 82-PH-1				
	5A	0	0	0
	5M-I488	2	2	3
	5M-T173	2	2	2-3
	5A + 5M	2	1[a]	1[a]
P. X euramericana				
cv. I-154				
	5A	1-2	1-2	2
	5M-I488	3	3[b]	4
	5M-T173	3	3	3[b]
	5A + 5M	2	2	2[b]
cv. I-214				
	5A	3	3	3
	5M-I488	2	2	2
	5M-T173	2	2	2
	5A + 5M	2	2	2
cv. 65/70				
	5A	1	2	3
	5M-I488	3	4	4
	5M-T173	3	3	4
	5A + 5M	2[b]	3	3
P. alba				
cv. Hickeliana				
	5A	0	0	0
	5M-I488	1[a]	1	0
	5M-T173	1a	1[a]	2
	5A + 5M	0	0	0

Superscript a indicates to presence of 1 or 2 small uredinia, while b indicates larger uredinia.

Table A7.5 Reaction of twelve cultivars of poplars to serially cultured race and racemix of M. medusae , involving natural virulent mutant, as assessed by infection type (0 - 4 scale). Race 4M-T173 from 6th generation was not included in the study.

	CULTIVAR	RACE	GENERATION 1	6	11
P. deltoides	cv. W-79/304				
		4B	1a	2	2
		4M-I488	2	2	2-3
		4M-T173	2	-	2
		4B + 4M	2	2	2
	cv. W-79/306				
		4B	1	1a	2
		4M-I488	2	2	3
		4M-T173	2	-	2
		4B + 4M	2	2	2
	cv. W-79/307				
		4B	3	3	3
		4M-I488	3	3	3
		4M-T173	3	-	2-3
		4B + 4M	3	2-3	2
	cv. 7-2				
		4B	3	3	2-3
		4M-I488	1	1	1
		4M-T173	1	-	0
		4B + 4M	2	1a	1
	cv. 7-4				
		4B	1	1a	2
		4M-I488	2-3	3	3-4
		4M-T173	2-3	-	2-3
		4B + 4M	3	2-3	2
	cv. 40-2				
		4B	0	0	0
		4M-I488	0	0	0
		4M-T173	0	0	0
		4B + 4M	0	0	0
	cv. 60/164				
		4B	0	2	2-3
		4M-I488	2-3	3	4
		4M-T173	2-3	-	3
		4B + 4M	2	3	3

APPENDIX 8

Infection Type (0-4 scale) of parental cvs 60/122 and T-173, and their F1 progeny to of seven races of *Melampsora medusae*.

	PARENTS		PROGENY																		
RACE	60/122	T-173	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1A	1	1	1	3	1	4	3	3	1	2	1	1	1	1	1	3	1	4		4	2
2A	3	1	1	4	3	4	4	1	4	3	4	4	4	3	2	3	1	4		4	3
3A	3	1	0	4	2		1		2	1	3	2	3							2	
4B	1	0		3	1		0	1	0	1	0	0	0		0	0	1	4	1		1
5A	1	0		4	1		0	1	0	2	0	1	0		1	0	1	4	1		1
5M	3-4	3-4	1				3	4	4	3	3	4				4	4			4	
7A	4	2-3	2				4	3	4	4	2	4				3	4			4	

	PROGENY																				
RACE	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
1A		4	3	4	1	1	4	4	4		3	2		4				1			1
2A		4	3	4	4	3	3	4	4		3	4		4				1			1
3A		4	0	2				4	1	4	1	1			2			1	0	2	3
4B		2	1		1	0		2	0				1	1	1	3	2	1		1	0
5A		2	1		2	0		2	1				1	1	1	4	2	2		1	1
5M	4	4	3	4	3	4		4		2	2	3		4		4		4	3	3	2
7A	4	4	3	4	4	4		4		4	2	3		4		2		4	2	3	2

	PROGENY																						
RACES	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61		
1A										1				1	1	1		2	1	1	1		
2A										3				4	1	3		4	1	4	1		
3A	1	2	2	3	4		2	1	2		2	2	2		1	1			2				
4B	1	1		1	4	1							1	1	0		1	0	0	0	0		
5A	1	1		1	4	1							1	1	0		1	1	0	3	0		
5M					4	2	3	2				3			4	4	3		4		4		
7A					4	3	2	3				2			4	4	4		4		4		

	PROGENY																						
RACES	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	**81**	82	83
1A	1	1		4	4	1	4	3	1	3	3	4		3			3	4		4	3		3
2A	1	3		4	4	4	1	4	1	1	1	4		1			1	4		3	4		3
3A		2		2	4				3		3	2	1		1	3	0	3		2	3	1	0
4B	0		2	3	2	0	1	1				0	0		0			1	0	0			
5A	0		2	3	2	0	1	1				0	0		2			1	0	0			
5M	4				4	4		4	2	3		3	4			4	3				0	3	4
7A	4				4	4		3	3	4		4	4			3	4				0	4	3

APPENDIX 9

INHERITANCE OF RESISTANCE TO *MELAMPSORA MEDUSAE* IN ADDITIONAL CROSSES OF *POPULUS DELTOIDES*

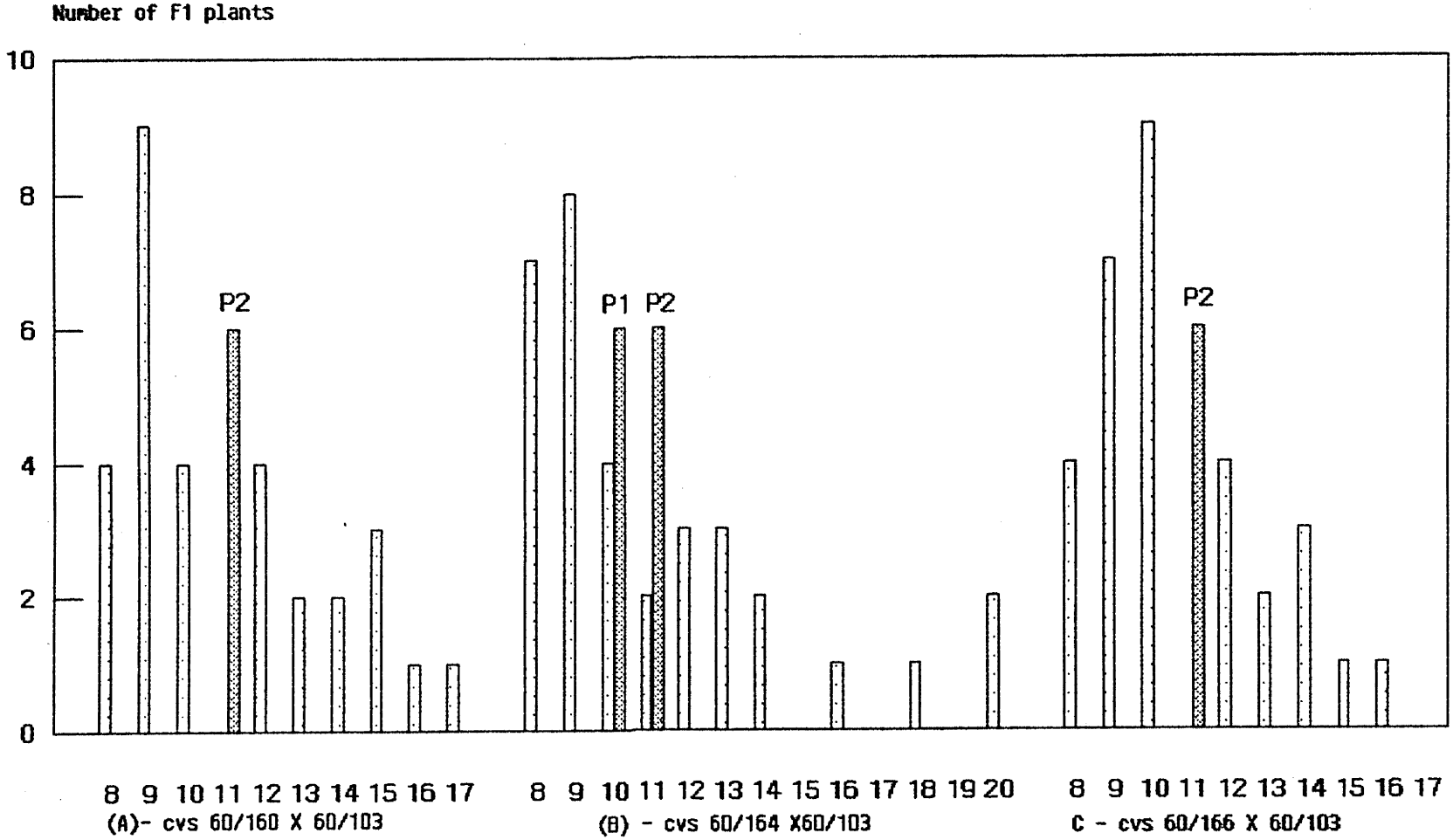
Studies on inheritance of qualitative and quantitative resistance to races of *M. medusae* in a cross of *P. deltoides* were reported in section III (Chapters 13 and 14). Here, analysis of resistance in a further three crosses of *P. deltoides* to race 5M of *M. medusae* is reported.

Three crosses, cvs. 60/160 X 60/103 (cross **A**), 60/164 X 60/103 (cross **B**) and 60/166 X 60/103 (cross **C**) were conducted during the spring of 1983. The cv. 60/103 was the pollen parent for all these crosses, and is susceptible to race 5M. The female parent cv. 60/164 was also susceptible while cvs 60/160 and 60/164 were completely resistant, to this race. The techniques for crossing, culture of plants, inoculation and incubation followed as detailed in Chapter 13. Many additional crosses with other cultivars were performed, but not included in the study because of poor seed setting or insufficient F1 plants.

Replicate leaf disks of the F1 progeny (5) and parents (10) were inoculated with urediniospores (3 mg) of race 5M, and incubated in 'standard environment' (section 2.4.1.). Because of the time constraint, the reaction of the plants to other races could not be assessed. Disease reactions assessed were, latent period to production of first uredinium (LP1) and uredinia produced per leaf disk (ULD). The ULD data were analysed employing square root transformed values.

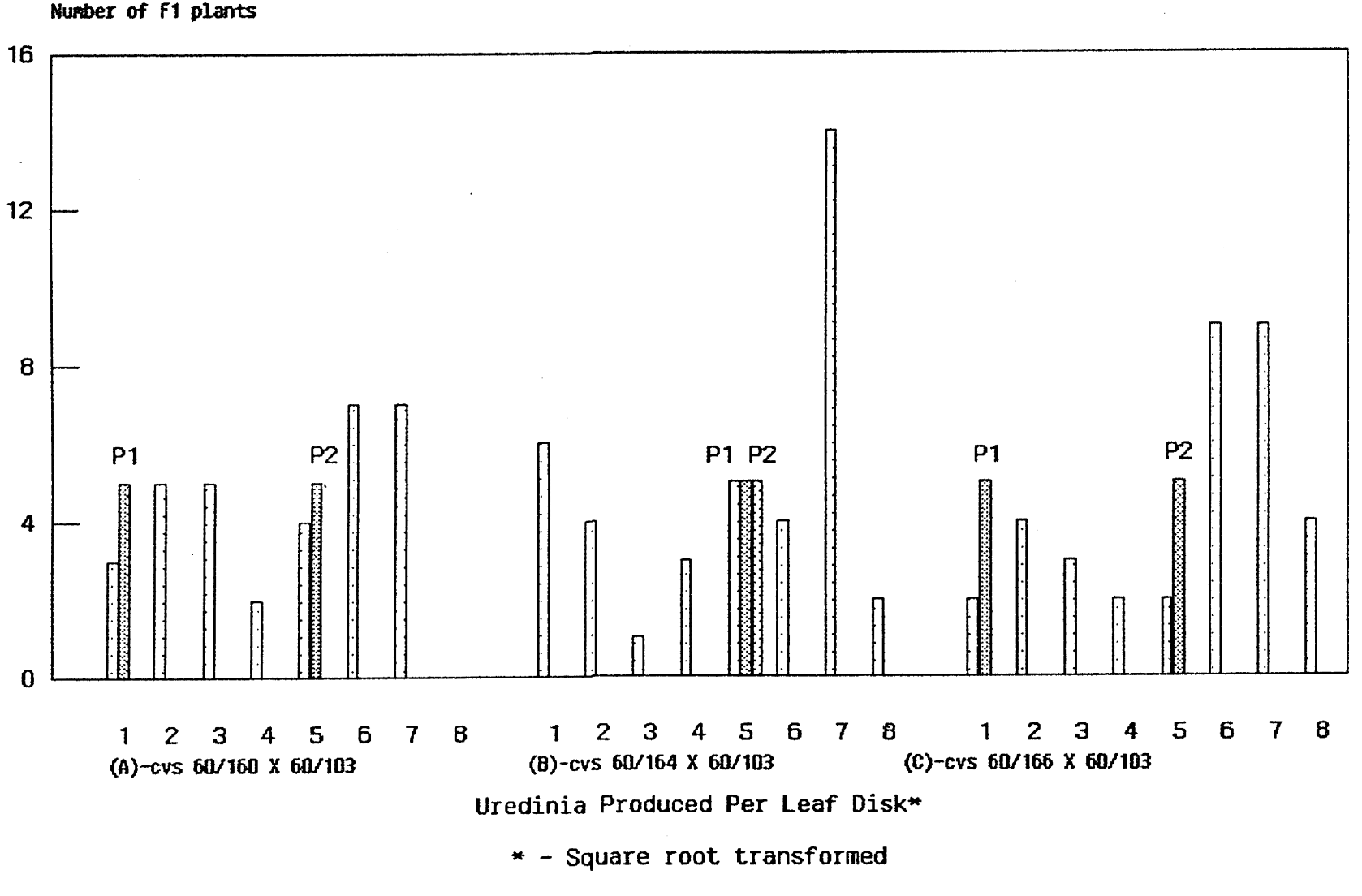
The frequency distribution of the F1 plants of the three crosses along with the parental positions, for LP1 (Figs A9.1) and ULD (Figs A9.2) are presented. As the cvs 60/160 and 60/160 were completely resistant (no uredinial production), the LP1 or ULD for these plants are not shown in Figs A9.1 and A9.2, repsectively. The distribution of the F1 plants for LP1 in all the three crosses were skewed, with an apparent

Fig. A9.1 Frequency graph for LP1 of F1 plants of 3 crosses of *P. deltoides* clones



Note : The parent P1 in crosses A and C was fully resistant

Fig. A9.2 Frequency graph for ULD* of F1 plants of three crosses of *P. deltoides* clones.



* - Square root transformed

dominance of the shorter, over longer, latent period (Fig. A9.1). The transgressive segregation towards longer latent period was evident in all the crosses, and more pronounced in cross B (Fig. A9.1), where a few plants with considerably very long LP1 were observed.

The distribution of the F1 plants for ULD was bimodal, which is indicative of qualitative or major gene interaction (Fig. A9.2). However, the continuity of the distribution observed within each class, is suggestive of the influence of polygenes. The skewness of the distribution was towards higher ULD, although a range of disease reaction from very highly susceptible to complete resistance could be observed. In cross B, where both the parents are susceptible (Fig. A9.2), few F1 plants with complete resistance and some plants with high quantitative resistance were observed. While this may be due to the recessive or epistatic nature of the resistance genes in these parents, it suggests that even susceptible parents can be useful contributors in breeding for disease resistance. The longer LP1 was usually associated with lower uredinia number and smaller pustules. However, as only one race of the pathogen was employed in this study, it is desirable to test the selected plants for reaction with other races, particularly in view of the pronounced race-specificity of the F1 plants of the other cross studied (Chapters 13 and 14).

The overall results are indicative of the interaction of both major and minor genes controlling resistance in these three crosses. Generally, the results resemble those reported in Chapter 14, where transgressive segregation and partial recessiveness of the resistance traits were observed.